QUALITY CHARACTERIZATION OF COOKIES DEVELOPED FROM A BLEND OF PRO-VITAMIN A CASSAVA (Manihot esculenta CRANTZ) AND BAMBARA GROUNDNUT (*Vigna subterranea (L.) Verdc.*) FLOURS

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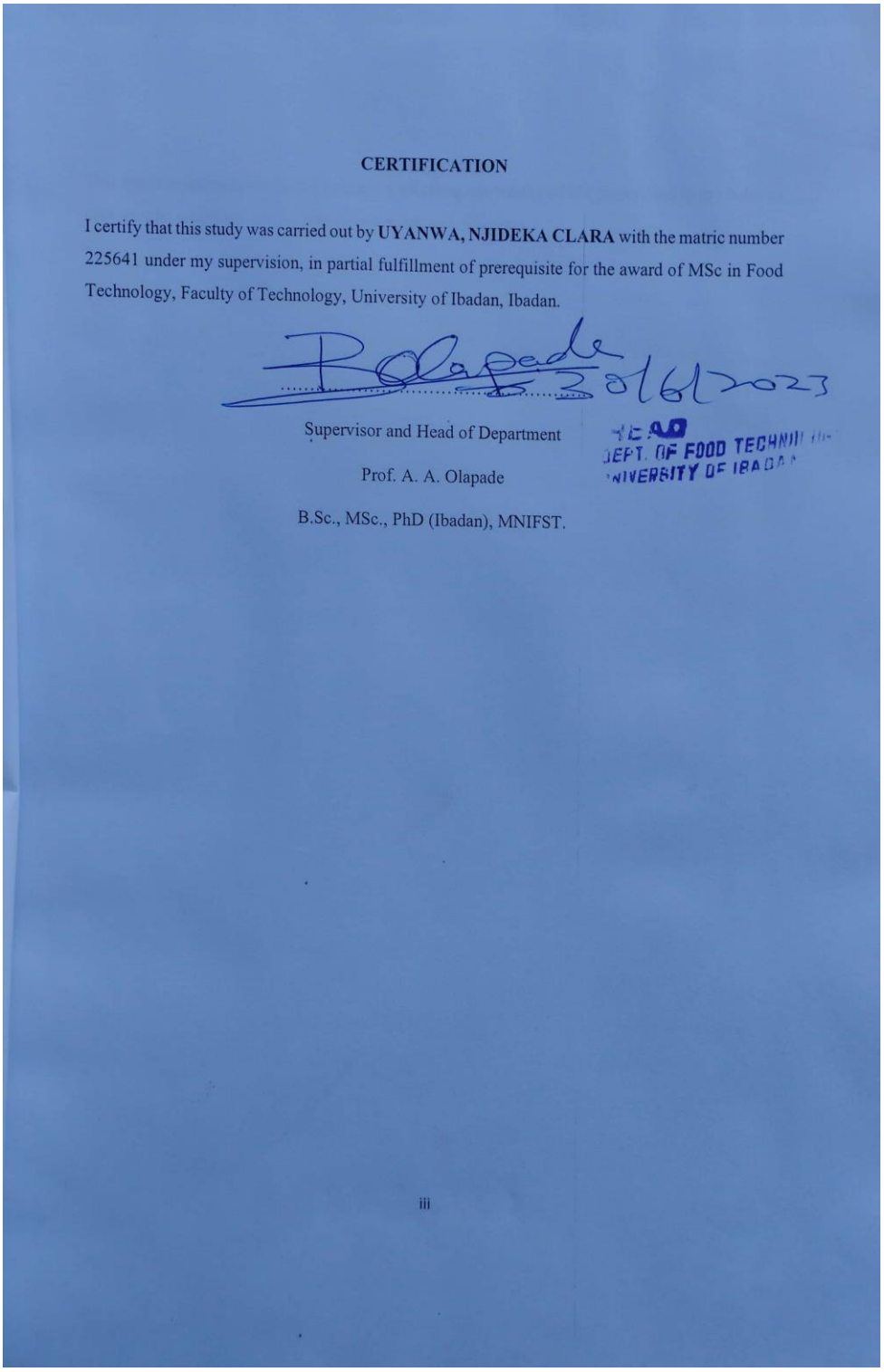
A Thesis submitted to the Department of Food Technology, Faculty of Technology, University of Ibadan, Nigeria

In partial fulfilment of the requirements for the award of the Degree of Masters in Food Technology.

JUNE, 2023

CERTIFICATION



CERTIFICATION

I certify that this study was carried out by **UYANWA, NJIDEKA CLARA** with the matric number 225641 under my supervision, in partial fulfillment of prerequisite for the award of MSc in Food Technology, Faculty of Technology, University of Ibadan, Ibadan.

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DEDICATION

This thesis is dedicated to God Almighty, for finding me worthy of His mercy, and to my beloved parents who taught me the virtues of hard work and diligence.

ACKNOWLEDGEMENT

I want to start by expressing gratitude to the Most High God for being with me at all times and has enabled me to finish this thesis.

I want to sincerely thank my supervisor and the Head of Department, Prof. A. A. Olapade for his diligent follow-up, advice, support from the early stages of the proposal to the final thesis write-up.

I want to specially thank my supportive and concern lecturers: Professor K. O. Falade, Professor R. Akinoso, Dr G. I. Arueya, Dr Olufunke O. Ezekiel, Dr A. O. Oladunjoye and Dr Folasade O. Adeboyejo for the steadfast support provided to me throughout my programme.

I am obliged to the entire non-teaching staff of Food Technology Department, University of Ibadan among whom are Mr B.O Oyedokun, Dr Y. A. Babarinde, Mr. P. O. Ayoola, Mr. M. J. Effiong, Mr. A. O. Austin, and Mrs. Sarah Olufunmilayo for their brilliant contributions, support and knowledge impacted during my laboratory analyses and throughout the programme. I also acknowledge with gratitude the very useful support of Mr T.A Akanji, Miss Hope Irabor and Mr J.O Olabode; thank you all for being there. Many thanks to my fellow MSc. Students for their assistance and insightful comments during the food sensory evaluation process.

I am thankful to my family, who saw hope in me and have never relented in any way to give me support, sincerely I am very grateful.

My genuine appreciation would fall short without naming the management of Federal University of Technology, Owerri for the fellowship to study. To the entire staff of the Department of Food Science and Technology, FUTO notably the Head of Department Prof (Mrs) G.C. Omeire and the immediate past Head of Department; Prof. C.I Onwuamanam. Thank you all for your support. I have the honour to express my sincere debt of gratitude to the immediate past Deputy Vice Chancellor, Research; Prof. Bede Anusionwu, for his guidance.

Worthy of mention is the contributions of the National Root Crops Research, Institute, Umudike, Umuahia; where the PVAC was sourced and in whose laboratory some of the analyses were carried out. I also acknowledge the very useful support of Mr Obi Ogbonnaya of CESLAB Global Services, Umuahia. I would like to express my boundless gratitude to my friends; Isioma

Obiodioka, Blessing Ogheneovo, Omotunde Fanimokun, Regina Okana, and others for their love, encouragement and continuous support during my study.

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ABSTRACT

Food-to-food fortification is a worthwhile approach to improve the quality of nutrition of populations. This study evaluated the quality characteristics of cookies produced from blends (%) of provitamin A cassava flour and Bambara groundnut flours (100:0, 80:20, 60:40, 40:60, 20:80 and 0:100). Functional properties, proximate, mineral, and beta-carotene content of the composite flours were evaluated while the colour parameters and antinutrient contents of the flours (main ingredients) were evaluated. The formulated cookies were evaluated for physical properties, colour parameters, proximate, mineral, beta-carotene content, antinutrient contents, sensory properties and in-vitro protein digestibility using standard methods. The results of the functional properties showed that composite flour improved in all the functional attributes investigated than the control samples.

Moisture content of the enriched cookies (5.75-6.74%) were within acceptable moisture level for cookies that will not support microbial proliferation. Increased substitution with Bambara flour resulted to increase in protein content (3.46-16.33%), ash content (2.64-4.26%) fat content (21.66-24.63%), and energy value (458.88-480.69 kcal/100g) respectively. Inclusion of yellow root cassava flour in the composite flours resulted to cookies with high beta-carotene content (47.06-91.39µg/100g). Physical properties of the cookies ranged from 8.31-9.02g weight, 4.08-4.53cm diameter, 0.54-0.68cm thickness, 6.04 - 8.46 spread ratio and 4.13-5.66kg breaking strength.

Anti-nutritional factors in the cookie samples were within permissible levels. Sensory analysis revealed that PVAC60:BG40 was the limit for moderate acceptance based on organoleptic characteristics. In-vitro protein digestibility revealed that the enriched cookies are highly digestibility (76.05-81.06%) and improved with increased Bambara flour substitution. This study revealed that the production of cookies from provitamin A cassava and Bambara flour resulted into products with improved nutrient composition, digestibility, sensory properties and acceptable/safe antinutrient limit which can be optimized for value addition. It is inferred that the nutrient-rich cookies made in this study would help treat vitamin A deficiency, protein energy malnutrition, and other conditions linked to poor/under nutrition.

Keywords: Pro-vitamin A cassava; Bambara groundnut; Enriched cookies; Composite flour; Under-nutrition; Food-to-food fortification; In-vitro digestibility

Word count: 320

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

The restricted leisure time and longer work hours of the global lifestyle have boosted the need for ready-to-eat foods (Filli *et al.,* 2013). In addition to being nutritionally superior and healthy, consumers desire snacks that taste, smell, and look appealing (Hazarika *et al*., 2013; Honi, 2016). The demand for ready-to-eat, convenient foods like biscuits, bread, cakes, chin-chin, cookies, and other pastry products has expanded as a result of the popularity of bakery goods (Okoye and Ezeugwu, 2019).

Cookies are small flat, sweet baked foods that are often made using flour, eggs, sugar, and either butter, cooking oil, or fat (Abayomi *et al*., 2013). They are inexpensive, wholesome, ready-to-eat baked snacks that come in a variety of sizes and shapes (Vijerathna *et al*., 2019). Cookies have gained popularity due to their inexpensive cost of production, convenience, long shelf life brought on by their low moisture content, and capacity to transport both essential and non-essential nutrients (Arise *et al*., 2021). Because they are widely accessible in nearby stores as ready-to-eat, affordable, practical, and delectable food goods, and also because of their creamy taste and low water activity which characterizes their extended shelf life, cookies are one of the most common baked items consumed by all age groups in numerous nations including Nigeria (Dauda *et al*., 2018; Ubbor *et al*., 2022). Most bakery products are produced using wheat as their main component because it is a cereal well-known for its gluten content (Kumar *et al*., 2011). However, a number of factors, including the negative economic impact of wheat importation on low- or non-wheat producing countries and the link between gluten sensitivity and wheat protein, have made it necessary to replace wheat with nutrient-dense locally grown crops like legumes, roots, and tubers (Arise *et al*., 2021).

Root and tuber crops such as yam and cassava are second only in importance to cereals as a global source of carbohydrates (Oladipo *et al.*, 2017). With cassava being the second most important tropical root crop in West Africa (Falola *et al.*, 2017; Oyeyinka *et al*., 2019). Cassava (*Manihot esculanta Crantz*), is a tropical crop that originated in northeastern Brazil. It is a dicotyledonous plant from the *Euphorbiaceae* family. Cassava is a crop with a high degree of adaptability because

it can withstand drought, needs little field preparation, and thrives in poor soil. Peel (10–20%) and edible fleshy section (80–90%) make up cassava. The roots are utilized as raw materials in numerous industries as well as for human consumption and animal feed. The flesh of cassava tubers is typically cream or white, and they do not have detectable levels of carotenoids (Okoye and Ezeugwu, 2019).

Vitamin A continues to be an essential part of human nutrition, as it helps with eyesight, cell differentiation, the synthesis of glycoprotein, reproduction, and general growth and development. Vitamin A Deficiency (VAD) and the seriousness of the effects, prevention, and treatment have turned into a worldwide concern (Okoye and Ezeugwu, 2019), since a population's health and development are seriously endangered by a shortage of these micronutrients, especially in developing nations (Bain *et al*., 2013). As a result, the creation and spread of varieties of yellow root cassava, also known as beta-carotene cassava, would support ongoing VAD prevention efforts by providing vitamin A through a common food that people consume every day. Assessing different food forms made from these newly developed crops for potential value addition to boost better and widespread usage of the crop becomes a need since the pro-vitamin A (beta-carotene) content, would enhance the consumers' nutritional standing (Okoye and Ezeugwu, 2019). Also, this will improve the availability of different vitamin A-based diets across the nation. For more than 2 billion people worldwide, cassava root is a staple crop that provides the majority of their starch and energy needs (Ferraro *et al*., 2016; Ubbor *et al*., 2022) but low in many other vital micronutrients, including proteins. The low protein content of cassava flour (CF) is one of the problems limiting its use in food production (Ajibola and Olapade, 2021).

In developing nations, malnutrition has long been characterized by low protein intake (Arrutia *et al*., 2020). Malnutrition and food insecurity increase a person's or a population's risk of contracting diseases. According to data, 22.8% of Nigeria's under-5 population suffers from severe stunting (Onuegbu and Ibeabuchi, 2021). Children who are undernourished cannot afford to learn effectively due to cognitive issues, vitamin shortages, stunting, and other long-term effects of previous under-nutrition (Ajibola, 2020). Food technologists are adjusting their focus to develop nutrient-rich products in response to rising demand for healthier diets. The simplest and most common way to make ready-to-eat food more nutrient dense is to fortify it with protein, vitamins, minerals, and other nutrients (Poornakala *et al*., 2020).

Food-to-food fortification (FtFF) is a cutting-edge method for regulating micronutrients using a food-based strategy. To increase the nutritional qualities of foods that are lacking in certain nutrients, the strategy involves adding foods that are high in micro- or macronutrients (Kruger *et al*., 2020; Vishwakarma *et al*., 2022). According to Honi (2016), this idea of increasing the consumption of foods high in essential micronutrients is more or less a sustainable strategy to reduce under-nutrition. Fortification with flour rich in proteins and a measurable amount of vitamins and minerals is therefore necessary to improve the nutritional content of foods designed from cassava flour (Ajibola and Olapade, 2021).

Bambara groundnut (*Vigna subterranea (L.) Verdc.*) is an underutilized African legume despite being Africa's third most significant legume after the common peanut/groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*) (Arise *et al*., 2022). Undernourishment can be reduced by using bambara groundnut, which is a source of protein, Carbohydrates, lipids, and minerals like iron. It is easily accessible, yet underutilized and poorly advertised in both domestic and foreign markets (Hillocks *et al*., 2012).

Composite flours, which are partly replaced with other natural nutritional elements (such as vitamins, minerals, proteins, dietary fiber, and antioxidants), have drawn the interest of manufacturers and consumers in recent years as a result of customers' desire for healthier food items (Lu *et al*., 2021). The term "composite flour" describes a blend of wheat flour and non-wheat flours from cereals, legumes, roots, and tubers, or it can also refer to a mixture of non-wheat flours (Okpala and Okoli, 2011). One of the many benefits of composite flour is its crucial role in compensating for vitamin deficiencies. It encourages the growth of high-yielding native plant species and improves domestic agriculture as a whole. It also keeps the hard currency safe (Okoye and Ezeugwu, 2019). There is currently scarcity of information on the utilization of composite flour made from yellow root cassava and Bambara groundnut flour in cookie making.

Worthy of note is that the main objective of FtFF is to deliver nutrient-rich foods to meet daily nutritional needs without sacrificing sensory appeal (Chadare *et al*., 2019; Vishwakarma *et al*., 2022). The purpose of this study is therefore to produce an acceptable and nutrient-dense cookie using PVAC and Bambara groundnut. The effect of replacement levels on cookie nutritional, physicochemical and sensory characteristics as well as the digestibility of the protein will then be

determined with a view to increasing the accessibility of vitamin A-based diets in the country in addition to the use of both Bambara groundnut and PVAC.

1.2 Statement of the Problem

There is an over reliance on using imported wheat flour in the food industry. Use of conventional flour from roots and tubers as well as legumes is a need in minimizing the economic impact of wheat importation.

Currently, many food industries voluntarily include vitamin A, but we also require vitamin A and protein contents. Moreso, consumers with life-long intolerance to wheat gluten need ready to eat foods that will meet their needs. Although it is plausible to develop a nutritious product by combining PVAC and Bambara groundnut, the precise formulations to do so have not yet been determined. In light of this, the researcher aims to look into the likelihood of developing an acceptable, nutrient dense cookie using Pro-vitamin A cassava and Bambara groundnut flours.

1.3 Justification for the Study

As a result of several health issues linked to food consumption, such as celiac disease (a lifelong intolerance to wheat gluten characterized by inflammation of the proximal small intestine), world nutrition agencies have intensified their support for the consumption of functional foods. The focus of food research in the twenty-first century is therefore on creating goods that improve consumers' health. Fighting under-nutrition among children and the general populace at large will be made easier with the intensive use of locally grown crops as sources of energy, protein, vitamins, and minerals. Cassava is regarded as a significant staple food crop in Nigeria, hence, consuming this high beta-carotene cassava can aid in the fight against vitamin A deficiency, a significant public health issue that affects many regions of the world. Using PVAC with Bambara groundnut in a nutritious cookie in balanced ratios can therefore help lower protein-energy and micronutrient deficiencies. According to predictions by Bellamy (1998), between twenty and thirty percent of deaths in children under five could be avoided if they receive a healthy diet rich in protein and vitamin A.

The food industry can as well make a “high protein”, “gluten-free” claim about cookie and other food products in accordance.

Moreover, this will provide further information on the use of both PVAC tubers and Bambara groundnut and will encourage their use by the food processing industries, hence, fostering conservation, domestication, improvement and utilization of these locally produced underused species.

1.4 Objectives of the study

The overall objective of this study was to produce an acceptable, digestible, nutritious cookie from Pro-vitamin A cassava and Bambara groundnut.

The specific objectives of this study were:

1. To evaluate the nutritional value of cookies made with Bambara groundnuts and PVAC composite flours
2. To ascertain the consumer acceptability of the formulated cookies.
3. To investigate the digestibility of protein in the formulated cookie

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Background

The decades-long efforts to end hunger, malnutrition, and ensure food security in most of the world are being undermined by factors like rising population, poverty, underutilization of nutritionally dense indigenous crops, and persistent postharvest losses of agricultural products like fruits, vegetables, roots, and tubers (Adubofuor *et al*., 2016). Incorporating highly perishable crops such as cassava into commonly consumed dishes, as well as processing and using underutilized indigenous products like legumes, will not only address malnutrition and food insecurity but also reduce food waste (Adubofuor *et al*., 2016; Arise *et al*., 2019; Arise *et al*., 2017a).

2.2 Legumes

Legumes are comestible seeds belonging to the Leguminosae family, the second largest seed plant family with 600 genera and 13,000 species. The word legume comes from the Latin legere, which means "to gather," implying that the seeds are collected by hand. Legumes are common diets for several people in diverse parts of the globe. Its seeds contain as double as protein content of cereals in percentage and commonly comprise of more stable composition of essential/indispensable amino acids (Vijayakumari *et al*., 1997). The majority of proteins in legumes are storage proteins. Although legumes are high in protein, they represent only a small part of most people's diets. In the world, each person consumes an average of 21 grams of beans every day and 112 grams of meat. In addition to being a mainstay of most traditional diets, legumes are also low in greenhouse gas and water emissions, replenish the soil through nitrogen fixation, economical, and a reliable source of protein. The twelve major legume crops of the FAO commodity classification include soybean, followed by peanut dry beans, dry peas, chickpea, cowpea, fava bean, lentil, pigeonpea, miscellaneous beans, lupin, and Bambara bean (Semba *et al*., 2021).

Because of their great nutritional content and availability, legumes, especially Bambara, are used as functional components for the preparation of a variety of meals, either on their own or as components in other food products (Arise, 2016).

2.3 Bambara Groundnut

2.3.1 Description and Botanical Classification

Bambara groundnut (*Vigna subterraenea (L.)* verdc) (Plate. 2.1) is an uncomplicated to grow legume seed that belongs to the Fabaceae family, the faboidea subfamily, and the genus Vigna. The nuts come in both cultivated (*V. subterranea* var. *spontanea*) and wild (*V. subterranea* var. s*ubterranea*) variants (Murevanhema and Jideani 2013). The groundnut is an African native (Murevanhema and Jideani, 2013; Arise *et al*., 2015; Arise, 2016; Temegne *et al*., 2018) that has been underutilized even when it is considered to be the third-most significant legume after the very popular groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata*) (Adebowale *et al*., 2011; Hillocks *et al*. 2012; Arise 2016). In Nigeria, it is frequently occurring and is referred to as; "*Okpa*" (Igbo), "*Epa-roro*" (Yoruba) and "*Kwarur*u" or "*Gurjiya*" (Hausa) regionally (Abdulrashid and Hassan, 2021).

Bambara groundnut has been identified by Bamishaiye *et al*., (2011) as an intermediate, herbaceous, and annual plant. It is a hardy crop that has come to be recognized as an essential source of wholesome food when food is in short supply (Mbosso *et al*., 2020). This might be explained by its climate-smart traits, such as its pest tolerance (Tweneboah, 2000; Damfami and Oat, 2020), the capacity to fix nitrogen and to thrive in challenging environments like drought and poor soils (Mayes *et al*., 2019, Paliwal *et al*., 2020; Tan *et al*., 2020) even in regions with less than 500 mm of yearly rainfall (Damfami and Oat, 2020).

The protein composition of bambara (20.5-32.4%) is comparable to that of other pulses like cowpea, soybean, and groundnut, with a notable amount of essential sulphur-containing amino acids which are more abundant than those found in most legumes (Murevanhema and Jideani 2013; Arise *et al*., 2015; Arise, 2016; Temegne *et al*., 2018). The seed is known for being quite filling. Its nutritional energy (per 100 g) has been calculated to be between 367 and 414 calories, which is more than typical pulses like cowpea, lentil, and pigeon pea (Damfami and Oat, 2020) starches, ranging from 33-53%, with amylose between 16 and 35% (Halimi *et al*., 2019; Kobue-lekalake *et al*., 2022).

2.3.2 Origin and Ecology

Bambara groundnut is indigenous to Africa, more notably the north-eastern regions of Nigeria and northern Cameroon (West Africa) (Temegne *et al*., 2018; Yao *et al*., 2015). In reality, the popular name (Bambara groundnut) appears to have come from the Bambara tribe, the majority of whose members presently reside in Mali. Nonetheless, there is disagreement over the true source of Bambara groundnut's diversity or origin (Temegne *et al*., 2018).

2.3.3. Cultivation

With decent rainfall, Bambara groundnut planting season runs from October through November and into early December. The reported emergence time for bambara groundnut is 7–15 days (Damfami and Oat, 2020). Bambara groundnut is sown between June and July in Nigeria's Plateau State and it typically takes between three and six months to reach maturity, depending on the cultivar and the weather (AFF *et al*., 2011; Damfami and Oat, 2020). When the leaves begin to dry out, the pods are ready to be harvested (Damfami and Oat, 2020). When fully dried, the seeds are smooth, spherical, and 1 to 5 cm in diameter. About 130 to 174 days are needed for the crop to grow. Unlike most leguminous crops, this one is extremely adaptable and can withstand adverse conditions (Damfami and Oat, 2020).

2.3.4. Varieties of Bambara groundnut

Botanically speaking, bambara groundnut is referred to as *Vigna subterranea* (L.) Verdc. It comprises two botanical varieties, namely *V. subterranea var. spontanea* which includes the wild varieties and V. subterranea var. subterranea which includes, the cultivated varieties (AFF *et al*., 2011).

Bambara groundnut cultivars come in seven different kinds, which can be distinguished mostly by the color or design of its seeds. They include;

• The black variety; which is specifically known for its early maturity; with kernels typically small to medium-sized and are usually comprised of one seed

• Red: Unlike the black variety, the red variety exhibits late maturation with large kernels. It is a good yielder (produces well), but prone to decaying on the site

• Cream/black eye: Known for having big kernel and yields well.

• Cream/brown eye: A moderate kernel that yields well

• Cream/no eye: Tiny kernels and pods. It primarily produces one seed and has poor yields.

• Speckled/flecked/spotted: The colour purple is predominant. Little kernels and single-seeded pods are the norm.

• Brown: Kernels are of medium-sized to large-size with incessant change between light and dark brown (AFF *et al*., 2011).

In Africa, white and cream landraces with large seed sizes are preferred (Akpalu *et al*., 2013; Damfami and Oat, 2020). Most peasants in Nigerian cultivate cream-colored varieties with white eyes.

2.3.5. Chemical Composition and Nutritional Value of Bambara groundnut

2.3.5.1. Proximate Composition of Bambara groundnut

One hundred grams of the edible fraction of dry seeds comprises water (10.35 g), energy (367 Kcal), protein (18.8 g), fat (6.2 g), carbohydrate (61.3 g), fiber (4.8 g) and ash (3.4 g) (Damfami and Oat, 2020). Arise and Malomo (2021) reported proximate compositions (in percentages) for the two types of Bambara groundnut; for the wild variety (*Voadnzeia subterranean*) protein (32.40%), carbohydrate (51.79%), fat (7.35%), ash (5.78%), and fibre (2.68%); for the cultivated variety (*Vigna subterranean*) protein (20.60%), carbohydrate (56.51%), fat (6.60%), ash (3.25%), and fibre (6.34%) (Table 2.1). According some other researchers, Bambara groundnut comprises 24–25% proteins, 58–62% total carbohydrates, 5.9–6.1% fat, 3.4–3.7% crude fiber, 3.6–3.8% ash (Kaptso *et al*., 2015; Kobue-lekalake *et al*., 2022). Others reported 64.4% carbohydrate, 23.6% protein, 6.5% fat, and 5.5% fiber with abundance in minerals (Tan *et al*., 2020; Azman *et al*., 2019).

2.3.5.2 Vitamin and Mineral Composition of Bambara

Angeot *et al*., (2014) reported Bambara groundnut as a good source of Ca, K, Mg, P and Fe with concentrations high enough to be useful in the diet of consumers. The values (in g/100 g dry matter) of macro-minerals in Bambara groundnut are Ca (37-128), K (1545-2000), Mg (159-335), Na (16-25) and P (313-563). The micro-minerals include Cu (3.0-13.2), Fe (23.0-150) and Zn (13.9-77.0).

Table 2.1: Proximate composition (%) of different species of Bambara groundnut

Species	Protein	Carbohydrate	Fat	Ash	Fibre
Voadnzeia subterranean	32.40	51.79	7.35	5.78	2.68
Vigna subterranean	20.60	56.51	6.60	3.25	6.34

Source: (Arise and Malomo, 2021)

Generally, the most abundant minerals in Bambara groundnut are potassium, magnesium, phosphorus, zinc, and iron. Despite being a relatively good source of these minerals, it is unlikely that the dietary needs of individuals can be met through consumption of Bambara groundnut alone (Tan *et al*., 2020). The high potassium and low sodium contents in the Bambara groundnut are desirable to combat high blood pressure (Kobue-lekalake *et al*., 2022). β-carotene (10 μg), thiamin (0.47 mg), riboflavin (0.14 mg), niacin (1.8 mg) and ascorbic acid traces (Damfami and Oat, 2020). The quantity of these minerals is minute and are however mostly lost as a result of processing.

2.3.5.3 Phytochemicals

Phytochemicals like flavonoids and tannins are present in Bambara groundnut seeds. Typically, these compounds are present in the seed coats, and seeds with dark or red seed coats tend to have higher concentrations of these substances. Total phenolic compounds and seed coat darkening have been found to be positively correlated (Tsamo *et al*., 2018; Tan *et al*., 2020).

2.3.5.4 Other Important Functional Properties

Dietary Fiber

Resistant starch and non-starch polysaccharides, two forms of dietary fiber, are present in significant amounts in bambara groundnut (Yao *et al*., 2015). Bambara groundnut has a total dietary fiber content that ranges from 1.4 to 10.3%, with insoluble fiber making up a larger portion than soluble fiber (Azman *et al*., 2019). The maturity stage and processing techniques however affect the composition and concentration of dietary fiber (Yao *et al*., 2015). Bambara groundnut is a low glycemic index (GI) food due to its relatively high proportions of slowly digestible starch (SDS) and resistance starch (RS) compared to rapidly digestible starch (RDS), as well as the presence of dietary fiber, which slow down digestion and lower the postprandial glycemic response (Oyeyinka *et al*., 2017; Tan *et al*., 2020). However, non-digestible dietary fibers can bind to minerals and create a physical barrier to digestive enzymes, which can compromise the nutritional security of the diet by lowering the bioavailability of essential minerals (Rousseau *et al*., 2020; Tan *et al*., 2020).

2.3.5.5 Anti-nutritional Factors (ANFs)

Anti-nutritional factors are substances that lower the nutrient utilization of plants or plant products used as human diets and play a crucial role in regulating the usage of plants for people (Gemede and Ratta, 2014). These are unfavourable legume ingredients that could prevent the absorption and

use of vital minerals including calcium, magnesium, iron, and zinc, among others thereby contributing to mineral deficiency (Vasagam and Rajkumar, 2011; Qayyum *et al*., 2012; Ajibola 2020). They are typically toxic and may reduce the nutritious content of seeds by reducing the availability of minerals and the digestion of proteins. However, because they are heat labile, processing techniques involving heat generation render them inactive (Ndidi *et al*., 2014; Ajibola and Olapade, 2021).

Many ANFs have been found in Bambara groundnut, just like other legumes. They may have a detrimental impact on essential nutrient digestion and bioavailability. Condensed tannins, phytic acid, and trypsin inhibitor are among the most frequently observed ANFs in Bambara groundnut (Tan *et al*., 2020).

Several Bambara groundnut cultivars have revealed varying quantities of ANFs (condensed tannins, 0.0011-18.61 mg/g; phytic acid, 1.10-15.11 mg/g; trypsin inhibitor, 0.06-73.40 TI mg/g). Genetic and environmental factors, as well as extraction and analytical techniques, are thought to be responsible for these variations (Unigwe *et al*., 2018; Duodu and Apea-Bah, 2017). There have also been findings of other ANFs in Bambara groundnut, including oxalate, hydrogen cyanide, and saponins (Tsamo *et al*., 2018; Adeleke *et al*., 2017; Ndidi *et al*., 2014; Tan *et al*., 2020).

Although it has been demonstrated that anti-nutrients such tannin, phytic acid, and enzyme inhibitors limit the nutritional value of legume seeds (Murevanhema and Jideani, 2013). Nevertheless, the amount of tannin in Bambara groundnuts is considerably impacted by several processing techniques, including cooking, roasting, and autoclaving. In particular, dehulling, soaking, and boiling—while discarding cooking water—have been shown to be efficient in lowering the tannin concentration (Barimalaa and Anoghalu 1997; Yao *et al*., 2015).

2.3.6 Statistics of Bambara Groundnut Production in Nigeria

Although the yield potential is said to be about 3 t/ha, Bambara groundnut production in Africa is estimated to be about 0.3 million tons annually with an average of 0.85 t/ha (Hillocks *et al*., 2012; Nedumaran *et al*., 2015). With a mean production of 0.1 million tons, Nigeria is recognized as the leading producer of Bambara groundnuts, followed by Burkina Faso with 44,712 tons and Niger with 30,000 tons (Hillocks *et al*., 2012; Tan *et al*., 2020).

2.3.7 Food and Non-food Uses of Bambara groundnut

2.3.7.1 Food Uses

The main use of Bambara groundnut is for human consumption. During various phases of development, from immature to completely ripe, the seeds are eaten. The immature seeds can be eaten fresh, boiled, grilled, or combined with immature peanut or green maize for a meal (Bamishaiye *et al*., 2011). Due to the extreme hardness of the mature seeds, boiling must be done before any other kind of preparation. Ripe seeds are ground into flour, which is then used to make cookies or porridge by combining it with cereal and boiling it. In Zimbabwe, ripe dry seeds are also roasted, chopped, boiled, crushed and consumed as a relish; a cookie resembling a peanut is also made by roasting Bambara groundnuts. Moreover, the seeds may be dried and kept for a later time (De Kock 2016; Damfami and Oat, 2020).

The well-known "okpa" (steamed Bambara groundnut pudding), which is prepared in Nigeria from Bambara groundnut flour and red palm oil, is significant in boosting the dietary intake of protein and vitamin A among schoolchildren (Ayogu *et al*., 2017; Tan *et al*., 2020). According to reports, bambara groundnut is also used to bolster staples like ogi, a gruel made of maize. *Ogi* is a very popular cuisine that is primarily made of maize and eaten by both adults and children (Tan *et al*., 2020). In terms of its nutritional value and appearance, bambara groundnut milk is likewise thought to be the best for infants and nursing mothers. As a result, it is necessary to pay closer attention to this underutilized crop because there is a vast knowledge vacuum that has to be filled (Damfami and Oat, 2020).

The protein level of several food compositions has increased as a result of the utilization of bambara groundnut. It has been employed to enhance numerous staples, such as cookies, biscuits, breakfast cereals, extruded products, complementary food, doughnuts, vegetable milk and yoghurt, traditional foods such as fufu and so on (Arise *et al*., 2022; Arise and Malomo, 2020; Damfami and Oat, 2020; Nwadi *et al*., 2020; Olapade *et al*. 2014). It can be used in areas with high levels of iron (Fe) deficiency, particularly the red type, which has twice as much protein as the other varieties (Damfami and Oat, 2020). But most peasants in Nigerian cultivate cream-colored varieties with white eyes.

Bambara groundnut has been widely used to improve many staples including cookie, cookies, biscuits, breakfast cereal, pasta, traditional foods, cereal, and tuber flours as composites. These are

cheap sources of protein for low income earners because animal protein sources result in higher cost (Shankar, *et al*., 2018; Nwadi *et al*., 2020).

Processing methods have also been proven to have effect on the protein content and sensory properties of products enriched with Bambara groundnut. For instance, fermentation has been shown to improve the protein content of Bambara groundnut (Nwadi *et al*., 2020). In a review by Nwadi *et al*., (2020), processing methods such as roasting, germination, extrusion cooking have been suggested as ways of addressing the sensory problems associated with products enriched with Bambara groundnut.

2.3.7.1.1 Bambara Groundnut Flour

Bambara groundnut flour is one of the valuable products from Bambara groundnut. Generally, to process Bambara groundnut into flour, Bambara groundnut is sorted to remove extraneous materials such as stone and dirt and to separate insect-infested seeds from desirable ones. Broken, wrinkles and immature seeds are also sorted out. The seed coats are partially removed by splitting the seed in a milling machine or manually using mortar and pestle followed by winnowing to remove loosen test and converting the cotyledon into fine flour by milling several times using a milling machine. The flour is then sieved (Honi, 2016; Okoye and Ezeugwu, 2019).

2.3.7.2 Non-food Uses

According to Akpalu *et al*. (2013) and Damfami and Oat, (2020), black seeds are mixed with water to treat sick children, pulverized to treat skin rashes, and chewed to relieve swelling jaw ailments. White seeds are mixed with guinea fowl flesh as a remedy for diarrhoea. Pregnant women chew and ingest Bambara groundnut seeds to reduce the sickness brought on by pregnancy. Water and crushed BGN seeds are combined to treat cataracts (Olanipekun *et al*., 2019 ; Abdulrashid and Hassan, 2021).

Generally, it is a plant that is used medicinally to cure a variety of ailments, including abscesses, internal injuries, ulcers, infected wounds, diarrhea, anemia, kwashiorkor, epilepsy, cataracts, menorrhagia during pregnancy, nausea in pregnant women and venereal diseases. It also aids in the prevention of cardiovascular disease, eye illness, and colon cancer (Jideani and Diedericks, 2014). It possesses kaempferol, an antioxidant polyphenol that lowers the risk of several chronic diseases, including cancer (Temegne *et al*., 2018; Yao *et al*., 2015; Jideani and Diedericks, 2014) and to neutralize blood sugar (Damfami and Oat, 2020).

Its seeds are often utilized as pig and poultry feed and the green stems frequently fed to animals (Temegne *et al*., 2018).

2.3.8 Utilization Constraints of Bambara groundnut

According to reports, despite having a high protein level, the majority of food products made from Bambara groundnuts are not acceptable when it comes to taste and flavor (Yusuf and Ejeh, 2018; Nwadi *et al*., 2020). Its long cooking time and many constraints, including a lack of resources, a knowledge gap, social stigma, and a lack of governmental incentives, work against Bambara groundnut's ability to improve the world food system despite its remarkable nutritional and agroecological profile. The outlook for Bambara groundnut has however improved as a result of numerous research initiatives to overcome these obstacles, but more work is urgently needed to be done in order to reach its full potential. (Tan *et al*., 2020).

2.3.9 Digestibility of Bambara Groundnut Protein

Dietary protein quality is determined by the digestion of proteins and the absorption of their constituent amino acids (digestibility), as well as the usage of absorbed amino acids to promote whole-body protein synthesis (availability) (WHO, 2007; Semba *et al*., 2021).

During protein digestion, peptide bond hydrolysis releases H+, resulting in an automatic fall in pH. As a result, the faster decline represents faster or higher digestion rates, and this phenomenon is utilized as a measure of protein digestibility (Malomo and Aluko, 2015). The faster rate of protein digestion is associated with easier protease access to peptide bonds as a result of lower quantities of non-protein components or, in some cases, a drop in polysaccharides and phytate prior to digestion. Fibres have been shown to reduce protein digestibility due to non-specific interactions between proteins and dietary polysaccharides (Malomo and Aluko, 2015; Arise and Malomo, 2021).

Low digestibility of the protein found in legumes is one of the main drawbacks limiting their nutritional quality (Oyeyinka *et al*., 2019). Although pretreatments such as boiling, germination, and soaking have been shown to enhance the protein digestibility of Bambara groundnut (Adeleke *et al*. 2017; Oyeyinka *et al*. 2019). However, Nwadi *et al*., (2020) in a current review suggested investigation of the digestibility of products enriched with Bambara groundnut using in vivo and/or in vitro processes.

Most legumes are processed in different ways according to recipes and culinary traditions of the various regions (Oyeyinka *et al.,* 2017). Soaking is an important precursor to a number of processing methods such as germination, cooking and fermentation (Kaushik *et al*., 2010). It has been reported to cause reduction in levels of minerals, phytic acid and proteolytic enzyme inhibitors in soybean (a legume) (Oyeyinka *et al*., 2019).

In addition, thermal processing of foods alters their physical, biological and chemical properties leading to sensory, nutritional and textural changes (Palermo *et al*., 2013). Protein denaturation and starch gelatinization during wet cooking enhances digestibility and bioavailability of protein (Palermo *et al*., 2013). Furthermore, microorganisms are destroyed and most antinutritional factors peculiar to legumes are reduced in the process. However, Soetan and Oyewole (2009) and Wang *et al*., (2010) reported that long cooking time can reduce certain essential amino acids in some legumes.

This study therefore explored soaking of Bambara groundnut prior to conversion to flour with the aim of improving digestibility of the composite cookies as already attempted by Arise *et al*., (2022) in production of protein isolate from Bambara groundnut for use in noodle production.

2.4 Roots and Tubers

Root and tuber crops such as yam and cassava are second only in importance to cereals as a global source of carbohydrates (Oladipo *et al.,* 2017). However, cassava is the second most important tropical root crop in West Africa (Falola *et al.,* 2017; Oyeyinka *et al*., 2019).

2.5 Cassava

2.5.1 Description

Cassava is a starchy root vegetable and underground part of the cassava shrub, which has the Latin name *Manihot esculenta,* commonly called cassava, yuca, mandioca like potatoes and yams, it is a tuber crop (Fakir *et al*., 2010; Verma *et al*., 2022).

Cassava (*Manihot esculanta Crantz*) belongs to chief humid root crops (Liu *et al*., 2011) and has been found to be capable of tolerating the most hostile climatic circumstances which other food crops may not endure (Jarvis *et al*., 2012). It is a dicotyledonous plant belonging to the family *Euphorbiaceae*. Cassava comprises of the peel (10-20%) and the edible fleshy portion (80-90%).

It is drought tolerant, requires limited land preparation and grows well in poor soil, all these attributes make it an extremely adapted crop (Okoye and Ezeugwu, 2019). In addition, cassava is easy to cultivate and its roots can stay reserved in the soil for several months when the farmer's storage space is limited, thereby creating opportunity for extended harvest and sustained availability. It therefore fits description as a survival crop that can potentially secure food supply and sustain livelihoods of large populations in difficult times (Ayetigbo *et al*., 2018). Cassava roots generally possess a cream or white flesh color and do not contain a legible amount of carotenoids (Okoye and Ezeugwu, 2019).

Cassava is a staple food crop for more than half a billion people in the tropical and subtropical regions of the world and mainly used as food, feed and industrial raw material (Vimala *et al*., 2010; Falade, 2010; Ayetigbo *et al*., 2018). More than 800 million persons depend on cassava as main basis of their calories (Sowmyapriya *et al*., 2017). It is the sixth most important commercially cultivated food crop after wheat, maize, potato, rice and barley (Ayetigbo *et al*., 2018).

Cassava root is a high calorie food with a high percentage of carbohydrates (80–90% dry basis) consisting almost entirely of starch. Nonetheless, cassava root is relatively poor in other nutrients such as proteins, lipids, and vitamins (Ayetigbo *et al*., 2018). It is an essential substance crop and chief carbohydrate mostly eaten in numerous ways by people. This creates the basis for diverse varieties of cassava-based diets in some continents (Balagopalan, 2002; Taiwo, 2006). In urban areas, cassava consumption of poor households is double that of non-poor households. In rural areas, poor households' consumption of cassava is triple that of non-poor households. When dried, cassava is both conservable and transportable over long distances (Akinpelu *et al*., 2011; Verma *et al*., 2022). It therefore contributes enormously to worldwide food security and expected to partake more substantial part in forthcoming (Rosenthal and Ort, 2012).

2.5.2 Origin and Ecology

Cassava was first introduced to tropical Africa from America by the pioneer Portuguese traders in 1558 (16^{th} century) (Ujong and Fashakin, 2022). Sub-Saharan Africa, Asia, and South America are the largest cassava producers in the world (Ayetigbo *et al*., 2018) Presently, almost 50 % of the cassava production in the world comes from Africa, where it is used as a staple food due to food insecurity (Guira *et al*., 2017; Ujong and Fashakin, 2022). It is cultivated in the tropical regions and was originated from North-East Brazil (Okoye and Ezeugwu, 2019).

According to other reports, Cassava is a tropical crop and its center of origin is believed to be the Amazon region. It was already cultivated by South American indigenous populations (Demiate and Kotovicz, 2009). It has been reported that the crop originated from South America where it has been grown by the indigenous Indian population and was domesticated between 5000- and 7000-years B.C. (Olsen and Schaal, 2001; Akinpelu *et al*., 2011). However, it is now grown throughout the tropical world (Verma *et al*., 2022).

Cassava is very flexible with respect to planting dates and hence under irrigated conditions in the tropics, planting can be taken at any time of the year. Cassava production depends on a supply of quality stem cuttings. The multiplication rate of planting materials is very low compared to grain crops, which are propagated by true seeds. In addition, cassava stem cuttings are bulky and highly perishable as they dry up within a few days (Verma *et al*., 2022).

2.5.3 Botanical Classification/Variety

Cassava (Manihot esculenta Crantz) can be classified into two distinct classes - "sweet" or "bitter". These two common botanical types are classified based on the cyanoglycoside composition. Thereby categorising the tubers into "sweet" and "bitter" cassava. Sweet type encompasses low cyanoglycoside content (<140ppm) while, bitter type contains over 140ppm cyanoglycosides on dry basis (Falade and Akingbala, 2008). Both bitter and sweet varieties of cassava contain antinutritional factors and toxins, with the bitter varieties containing much larger amounts. This is not because it is higher in sugars than other varieties, but because it is less poisonous. It may be classified into sweet and bitter based on the level of cyanogenic glucoside in the tissue (Uchechukwu-Agua *et al*., 2015). The cyanoglycosides are uniformly spread all over the sweet. For bitter cassava, the cyanoglycosides are mainly situated under cassava peel which can be easily removed during peeling, hence, excessive cyanide can be reduced during cassava processing (Falade and Akingbala, 2008).

Cassava contains large quantities of cyanide compounds, which must be processed out of the tubers before they can be safely eaten. The sweet variety of cassava has fewer of these compounds, and does not require as much processing. Sweet varieties also produce higher yields, has lower total carotenoid contents (2.08-4.38 μg g^{-1}) (Araujo *et al*., 2019). Sweet varieties can be eaten raw, boiled, or cooked without prior processing, bitter varieties are needed to processed to reduce risk of residual cyanogen prior to consumption (Chiwona-Karltun *et al*., 2002; Verma *et al*., 2022).

Bitter cassava is very similar in cultivation and general appearance to sweet cassava, but produces much higher quantities of cyanide compounds. It can be visually very similar to the sweet but require careful processing to make the flour safe to eat. 'Bitter' cassava had higher starch and sugar contents than the group of 'sweet' cassava (Araujo *et al*., 2019). The presence of cyanide in cassava constitutes a clear threat to health, unless these compounds are removed before the cassava is consumed. Unprocessed cassava is toxic enough to cause death, but insufficiently processed cassava will also cause mortality over a period of time, especially when quality protein is absent from the local diet. There are several methods of removing the cyanide from cassava. Simple drying reduces the level of cyanide, though this may not be adequate to make it safe for consumption. Soaking the roots in water first, to leach out cyanide, produces a safer starch. So does fermenting the roots, either whole, shredded or in pieces, before drying. Roasting the tubers, or boiling them in multiple changes of water, will also reduce the cyanide content (Verma *et al*., 2022).

Research has however shown the diverse discrepancies in level of cyanoglycosides from various cassava varieties irrespective of their classification as sweet or bitter varieties (Raji *et al*., 2007; CIAT, 2007).

2.5.4 Nutritional Composition of Cassava

Nutritional quality of cassava differs depending on varieties, stage of the plant, topographical setting and ecological situations (Gil and Buitrago, 2002). The roots are the main portion of the plant to be used with a typical composition range of: moisture 62 to 66%, starch 28 to 33%, sugars 0.4 to 1.2%, protein 0.4 to 1.5%, dietary fibre 1.4 to 1.6%, fat 0.1 to 0.3% and ash 0.5 to 1%. Iron and vitamin A are considered to be low (Verma *et al*., 2022). The moisture content of roots according to other reports ranged from 60.3 to 87.1% (Padonou *et al*., 2005; Zvinavashe *et al*., 2011). Its flour has a moisture content that differs from 9.2-12.3% (Charles *et al*., 2005) and 11-16.5% (Shittu *et al*., 2007a). The crude protein, lipid, fibre and ash contents of fresh cassava roots are low (0.9, 0.3, 0.5 and 0.4 g/100 g, respectively (Adepoju *et al*., 2010). Usually fibre content does not exceed 1.5% in fresh root and 4% in root flour (Gil and Buitrago, 2002; Agbemafle, 2019). It is also low in some vitamins and mineral contents (Charles *et al.*, 2005). According to Gil and Buitrago (2002), Cassava roots contain about 0.5% fat and good source of calcium and vitamin C of about 16-35 mg/100 g and 15-45 mg/100 g, respectively.

Other micronutrients of more recent significance and interest in cassava are pro-vitamin A carotenoids and vitamin C. Vitamin C, which is important for mineral absorption in the gut, is found in relatively higher amounts than carotenoids in fresh cassava (Ayetigbo, *et al.,* 2018). Among more than 500 cassava root lines evaluated by Chavez *et al*., (2000), vitamin C averaged 9.5 mg/100 g in fresh cassava although it is much more susceptible to losses during processing than carotenes. Minimal processing of cassava by methods susceptible to oxidation is recommended, if some vitamin C is to be retained. However, much of the processing techniques used for converting cassava to edible, safe food cannot guarantee its retention (Ayetigbo, *et al.,* 2018).

According to Zvinavashe *et al.,* (2011), cassava plant produces more carbohydrate by mass compared to other principal food crops in the same ecological situations. Its roots are functional energy reserve with high carbohydrate composition of about 32 to 35% and 80 to 90% on fresh and dry basis respectively. According to Charles *et al.*, (2005), it comprises of low protein content of about 1-2% that makes it mainly carbohydrate-based food. Due to its deficiency in protein, it is poor basis of a few indispensable amino acids namely tyrosine, methionine, phenylalanine, lysine and tryptophan (Falade and Akingbala, 2010). Cassava roots which are particularly poor in sulfur-rich amino acids are, however, relatively plentiful in acidic and basic amino acids such as glutamic, aspartic and arginine (Gil *et al*., 2002; Verma *et al*., 2022). In view of this, it needs fortification with protein enrich flour in preparation of balanced food.

Cassava is not particularly rich in all mineral nutrients; hence, diets based on cassava alone may not fulfill adequate mineral nutritional requirement in humans (Ayetigbo, *et al.,* 2018)

Carotenoids are important for healthy body metabolism and disease prevention. Of the carotenoids found in yellow-flesh cassava roots, β-carotenes are present in higher concentrations than other carotenoids involved in the biosynthesis of vitamin A (Aniedu and Omodamiro, 2012).

Biofortified provitamin A cassava is a type of cassava that is the result of cross-breeding. This type of cassava has a high level of carotene, is very resistant to disease, and can give children under 5 and pregnant women up to 40 % of the vitamin A they need every day (Ayetigbo, *et al.,* 2018; Bouis *et al.,* 2011; Ujong and Fashakin, 2022).

Cassava roots have calcium, iron, potassium, magnesium, copper, zinc, and manganese contents comparable to those of many legumes, with the exception of soybeans. The calcium content is relatively high compared to that of other staple crops and ranges between 15 and 35 mg/100 g edible portion. The vitamin C (ascorbic acid) content is also high and between 15 to 45 mg/100 g edible portions (Charles *et al*., 2004). Cassava roots contain low amounts of the B vitamins, that is, thiamin, riboflavin, and niacin, and part of these nutrients is lost during processing. Usually, the mineral and vitamin contents are lower in cassava roots than in sorghum and maize. The protein, fat, fiber, and minerals are found in larger quantities in the root peel than in the peeled root. However, the carbohydrates, determined by the nitrogen-free extract, are more concentrated in the peeled root (central cylinder or pulp). Thus, cassava roots are rich in calories but low in protein, fat, and some minerals and vitamins. Their nutritional value is, consequently, lower than those of cereals, legumes, and some other root and tuber crops (Montagnac *et al*., 2009a).

2.5.5 Yellow-fleshed versus white fleshed cassava varieties

In the regions where cassava is cultivated, the flesh color is traditionally white. However, other colored-flesh cassava variants (yellow, orange, cream, and red) have emerged to challenge this supposition (Ayetigbo *et al*., 2018).

There are many varieties of both white and yellow-fleshed cassava. The yellow-fleshed cassava is referred to as Pro-vitamin A cassava (PVAC). These varieties are increasing however due to continuous research. The result of the recent researches on cassava gave rise to the new breed of PVAC varieties (Ajibola, 2020). According to Kulakow and Egesie (2012), development of cassava varieties is the main productive insight which could enhance the nourishing status of individuals living on cassava-based diets.

In the yellow-fleshed cassava the major carotenoid pigment present is β-carotene. It has an important role as a principal pre-cursor of pro-vitamin-A which is involved in vision, cell differentiation, synthesis of glycoprotein, reproduction and overall growth and development of bones (Vimala *et al*., 2010)

Flesh color and culinary quality have therefore become vital in the selection of cassava for food (Vimala *et al*., 2010). White-flesh (and other dull colored) variants have negligible carotenoid content compared to yellow-flesh variants. Variants with deeper color intensity have a higher

carotene content. Visual differences also exist between root and flour of white-flesh and yellow-flesh variants, but not for the starch (Ayetigbo *et al.*, 2018).

As regards protein, protein content is significantly higher in the flour of white-flesh cassava variants than in the flour of yellow-flesh variants (Maziya-Dixon *et al*., 2005).

Cassava roots generally have a high moisture content, which can differ with variants. The moisture content of the respective root, flour and starch of yellow-flesh cassava variants is reported to be higher than for those of white-fleshed variants (Aniedu and Omodamiro, 2012; Ukenye *et al*., 2013) (Proximate composition of cassava root from white-flesh and yellow-flesh variants is shown in Table 2.2). Despite the relatively higher amount of moisture in yellow-flesh cassava roots, they tend to store better after harvest than their white-flesh counterparts, perhaps due to the additional anti-oxidative effect of carotenoids present (Chavez *et al*., 2000; Ayetigbo *et al*., 2018). This beneficial property could be appreciated by farmers, thus encouraging sustainable post-harvest practices and processing. Moreover, higher moisture content in the flour of yellow root cassava implicates lower starch content of the flour compared to the white fleshed variant according to the assertion by Aniedu and Omodamiro, (2012) . The starch content varies between 60.34 to 86.79 % of the mass of white-flesh cassava and 69.90 % of biofortified yellow-flesh cassava (Ayetigbo *et al*., 2018).

In a bid to sustain production of improved cassava variants in Nigeria, six yellow-fleshed variants were released under the IITA-HarvestPlus Project between 2011 and 2014 (Ayetigbo *et al*., 2018). The first set of released variants had a ß-carotene content of 6–8 µg/g on fresh weight basis, while the second set of variants introduced had an average ß-carotene content of about 10 µg/g on fresh weight basis. Several clones of yellow-flesh variants have been under investigation to select the most suitable traits for release. A total carotenoid content of almost 25 µg/g has been attained in South American variants (Sánchez *et al*., 2010; Ceballos *et al.*, 2013; Ayetigbo *et al*., 2018). Sustained efforts are ongoing to develop variants with up to 15 µg/g of ß-carotene (Saltzman *et al*., 2013).

Table 2.2: Proximate composition (%) of cassava root from white-flesh and yellow-flesh variants

Flesh colour	Moisture Content	Protein	Lipid	Fiber	Ash
White	57.46–75.91	2.07–7.92	0.02–3.66	0.62–4.92	0.33–1.1
Yellow	66.19–73.49	2.75–8.10	0.29–3.2	1.07–2.14	0.55–1.04

Source: (Ukenye *et al*., 2013; Ayetigbo *et al*., 2018)

Biofortified yellow-flesh cassava root, flour and starch has many similar physicochemical and functional properties as found for those of white-flesh cassava, and can, therefore, serve as a substitute in any products derived from white-flesh cassava root, flour and starch.

This flexibility in utilization is not possible for white-flesh cassava when pro-vitamin A nutrition is of concern, making this additional advantage of nutrition a case for sustained utilization and consumption of biofortified yellow cassava as food (Ayetigbo *et al*., 2018).

Most biofortified yellow-flesh cassava variants are sweet tasting, containing mild-to-moderate toxic cyanogenic glucosides compared to the majority of white-flesh variants (Talsma, 2014).

Post-harvest storage of biofortified yellow-flesh cassava is more sustainable, due to its robust and longer shelf-stability (Chavez, *et al*., 2000; Talsma, 2014) compared to white-flesh cassava. This implies it can secure longer availability periods, while the farmer awaits or is engaged in a new planting cycle.

Harvesting and post-harvest handling of some variants of biofortified yellow-flesh cassava has been reported to be less tedious than for white-flesh cassava. Farmers' response to surveys reveal they were easier to harvest and peel (Gonzalez *et al.,* 2011) than conventional white-flesh cassava

In Nigeria, value-addition has been achieved in using yellow-flesh variants in producing gari instead of the traditional practice of adding red palm oil to the white cassava granules to improve the vitamin A content of the food. Not only are production costs reduced, but a healthy form of fortification is also acquired by the yellow-flesh variants with yields close to the white-flesh ones. From a culinary and health/nutrition perspective, adding palm oil may not be acceptable in gari due to phase separation in water-based processes as well as for cholesterol-related issues. The acceptability of yellow-flesh variants is promising among farmers and consumers, as they are more stable after harvest than their white-flesh counterparts (Ayetigbo *et al*., 2018).

2.5.6 Antinutritional Factors

Although cassava has been reported to have some nutritional composition, it also contains antinutrients and toxic substances that interfere with the digestibility and the uptake of some nutrients. Nevertheless, depending on the amount consumed, these substances can also bring benefits to humans (Montagnac *et al*., 2009a).

Cyanide is the most toxic factor restricting the consumption of cassava roots and leaves. Indeed, cassava, particularly its bitter varieties, has a cyanide level higher than the FAO/WHO (1991) recommendations, which is < 10 mg cyanide equivalents/kg DM, to prevent acute toxicity in humans. Cassava root parenchyma has a range of 10 to 500 mg cyanide equivalents/kg DM (Siritunga and Sayre 2003); this is much higher than what is recommended. Several health disorders and diseases have been reported in cassava-eating populations. Consumption of 50 to 100 mg of cyanide has been associated with acute poisoning and has been reported to be lethal in adults. The consumption of lower cyanide amounts are not lethal but long-term intake could cause severe health problems such as tropical neuropathy, glucose intolerance etc (Montagnac *et al.*, 2009a). Nevertheless, reports have shown that age, variety, and environmental conditions influence the occurrence and concentration of HCN in various parts of the cassava plant and at different stages of development, respectively (Charles *et al.*, 2005). Presently in Nigeria, grating/crushing is being promoted in production of high quality cassava flour (HQCF) because it leads to the production of flour with negligible amounts of residual cyanide contents after drying (Adebowale *et al*., 2011). The joint FAO/ WHO Food Standards Program Codex Committee on Contaminants in Foods (JECFA) 3rd Session held in the Netherlands in 2009 concluded that a level of up to 10 mg HCN/kg in the Standard for Edible Cassava Flour (CODEX STAN 176-1989) was not associated with acute toxicity (Adebowale *et al*., 2011).

Phytate (inositol hexakisphosphate) is another compound found in high abundance in cassava, with approximately 624 mg/100 g in roots (Montagnac *et al*., 2009a). It is a storage form of phosphorus which is found in plant seeds and in many roots and tubers (Charles *et al*., 2005). Phytic acid has the potential to bind calcium, zinc, iron and other minerals (Charles *et al*., 2005). It may also bind proteins preventing their complete enzymatic digestion (Montagnac *et al.*, 2009a). On the other hand, phytate may play an important role as an antioxidant by complexing iron and thereby reducing free radical generation and the peroxidation of membranes, and may also act as an anticarcinogen, providing protection against colon cancer (Charles *et al.*, 2005; Montagnac *et al*., 2009a).

Others such as tannin, trypsin inhibitors, saponin, and oxalate are usually found in the leaves (Montagnac *et al*., 2009a).

Worthy to mention is that different processing techniques are relatively effective in removing cyanide from cassava and generally reduces part of its antinutritional value, especially those involving grating and crushing. The efficiency of the technique however depends on the duration of the process. But can also affect nutritional value through modifications and losses of dietary nutrients. Processing cassava into ready-to-eat products is therefore necessary to remove cyanogens and other antinutrients (Montagnac *et al*., 2009b, 2009a).

2.5.7 Statistics of Cassava Production

More than 291 million tons of cassava were produced worldwide in 2017, of which Africa accounted for over 60%. In 2017, Nigeria produced 59 million tons making it the world's largest producer (approximately 20% of global production) with a 37% increase in the last decade (FAO, 2017; Verma *et al*., 2022).

The worldwide production of cassava amounted to 278 million metric tons in 2018, out of which Africa's share was about 61% shown in Fig. 2.1. Nigeria's share of world production had risen to 21.5% of world production by 2018. FAO projects that by the year 2025; about 62% of global cassava production will be from sub-Saharan Africa (FAOSTAT, 2020).

2.5.8 Processing of cassava

Cassava is safe for consumption only after undergoing appropriate processing, which may include one or a combination of some treatments such as boiling, frying, fermenting, drying, baking, or size-reduction, all of which contribute to reducing cyanogenic glucosides and improves the palatability of products that are acceptable to consumers (Montagnac *et al*., 2009b; Ayetigbo *et al*., 2018). However, the downside of these processing operations is often a resultant reduction of nutrients, or conversion to other forms than the original nutrients. For instance, production of flour and other foods from cassava often leads to a loss of vital micronutrients (Ayetigbo *et al.*, 2018).

2.5.8.1 Cassava Flour (CF)

Cassava flour is a valuable product obtained from cassava roots after processing. Flour extraction from cassava tuber depends on reduction of moisture (Fakir *et al.*, 2012). Generally, to produce the flour, cassava root is peeled, washed, chipped, milled, pressed to expel most of the toxic liquor, dried, fine-milled, and sieved. It is relatively cheap to produce traditionally. Industry-grade high quality cassava flour, however, requires improved processing inputs, which may add to the costs

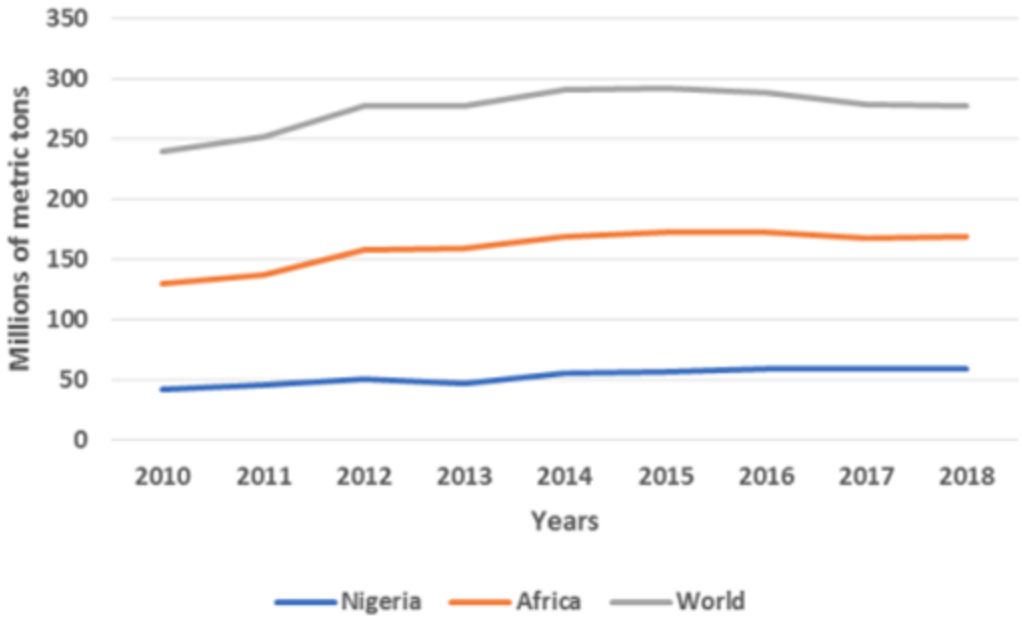

Fig 2.1: Production of cassava production between 2010 and 2018 (FAO, 2020)

(Ayetigbo *et al*., 2018). Cassava flour (CF) refers to the dry, fibrous and free-flowing particulate product obtained from cassava roots (Shittu *et al*., 2016). The main advantage of this flour Cassava flour that it is a grain-free and nut-free flour and is naturally gluten free and a great flour to use in baking and cooking therefore people who avoid gluten can use it as a replacement for wheat flour in term of taste and texture. Cassava flour does absorb more liquid than wheat flour (Verma *et al*., 2022).

2.5.8.1.1 High Quality Cassava Flour (HQCF)

High quality cassava flour is described as snowy, even, un-fermented and unscented cassava flour that is quickly processed within 24 h from fit cassava roots planted within ten to twelve months after cultivation. It has potential application in the production of composite flours with wheat flour and non-wheat flours for production of high-quality foods (Dziedzoave *et al.,* 2006).

The main difference between the traditional cassava flour and HQCF is the absence of fermentation which produces a low pH and acidic taste that are unwanted for industrialized foods. Therefore, production needs a firm obedience to upright manufacturing protocols to be able to acquire high quality end products of appropriate makings. The tubers should be of good quality, with absence of rot and must be harvested within ten months to a year after planting (Dziedzoave *et al.,* 2006; Ajibola, 2020). Tubers of over a year after planting minimize flour productivity (Apea-Bah *et al.,* 2011; Ajibola, 2020).

Due to its special properties such as its clear appearance, low off-flavor tendency and ideal viscosity, HQCF is regarded as a vital ingredient in the food industry (Ayetigbo *et al*., 2018)

2.5.9 Uses of Cassava

Presently more than 60% of cassava produced is used for industrial purposes, 30% is used for animal feed and only 10% is used for human food (Verma *et al*., 2022).

Though tuber is the main product of cassava plant, its young branch and leaf is also edible both for human and animal (Fakir *et al*., 2010). It is an important dietary staple in many countries with in the tropical regions of the worlds (Verma *et al*., 2022) where it provides food for more than 800 million people (FAO, 2007). Cassava root can be consumed raw as a snack or just after boiling like sweet potato (Fakir *et al.,* 2012). As a subsistence crop, it is the third most important carbohydrate food source in the tropics after rice and maize, providing more than 60% of the daily

calorific needs of the populations in tropical Africa and Central America (Verma *et al*., 2022). Cassava is the second most important staple food crop in sub-Saharan Africa, providing an average of 285 cal day^{-1} per person (FAO, 2000). It is the chief source of dietary food energy for the majority of people living in the lowland tropics, and much of the subhumid tropics of West and Central Africa (Oladunmoye *et al.,* 2014).

The utilization pattern of cassava however varies from one country to another. For instance, In Nigeria, about 90% of the country production is consumed (IITA, 2010). They are also processed into varieties of foods such as gari, fufu, lafun, cassava chips, cassava flour, cassava bread, cassava cookies etc. Research has also led to the production of high-quality cassava flour (HQCF), and fufu paste to mention but a few (IITA, 2011; Ajibola, 2020).

Cassava yield creates essential ingredients for animal feedstuff and can serve as a cheap source for formulating feeds for some animals. The starch produced from cassava processing has been used in making alcoholic drinks and preparation of lubricants (Echebiri and Edaba, 2008). Ethanol, biofuel are also products of cassava (FAO, 2018).

Cassava flour is a grain free and gluten free baking flour that is great for cooking and baking. It is used directly in different ways or as a raw material for further processing, and its unique properties also suggest its use for specialty markets such as baby foods and non-allergenic products (Verma *et al*., 2022). Different products derived from cassava root (Fig. 2.2)

2.5.10 Cassava Utilization Constraints

Cassava roots are high in carbohydrates but low in many other vital micronutrients, including proteins. The low protein content of cassava flour (CF) is one of the problems limiting its use in food production (Ajibola and Olapade, 2021). Because of this, research is ongoing and considerable efforts have been made to improve protein in cassava through food-to-food fortification (Ajibola, 2020; Ajibola and Olapade, 2021; Okoye and Ezeugwu, 2019; Ujong and Fashakin, 2022).

Yellow-flesh cassava, and other biofortified cassava have considerable potential in alleviating food insecurity in developing countries (Ayetigbo *et al*., 2018).

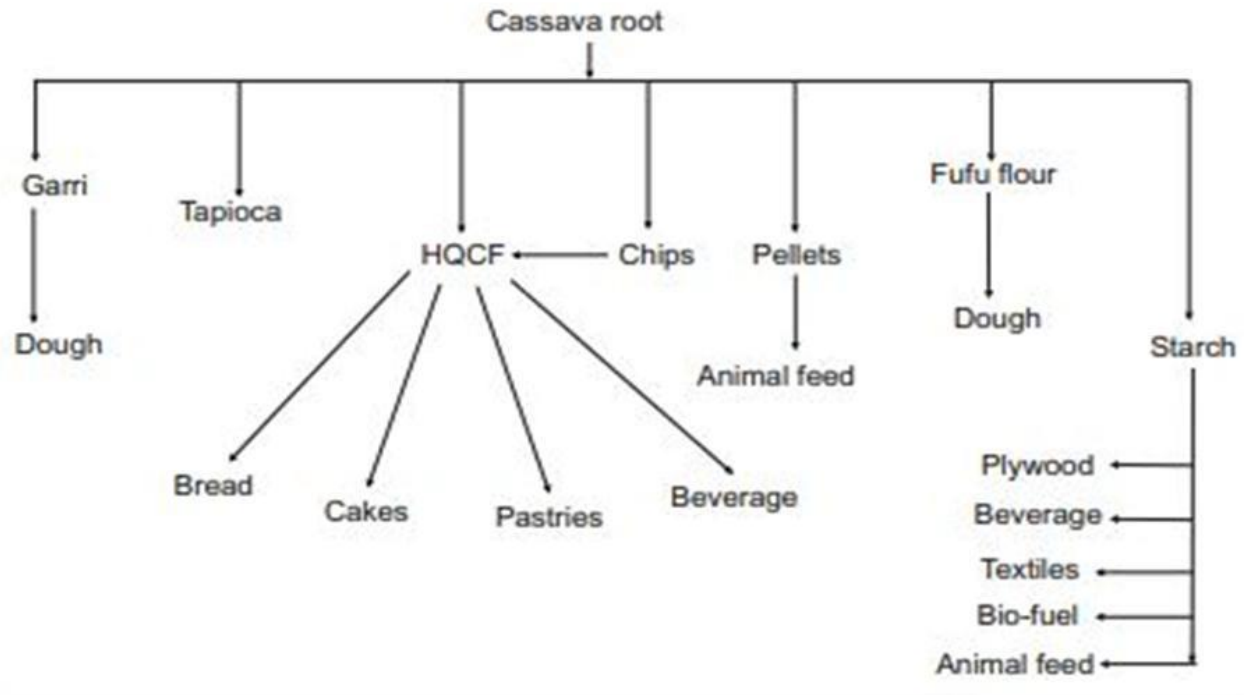

Fig 2.2: Different products derived from cassava root (Montagnac *et al*., 2009a; Falade and Akingbala, 2010; Verma *et al*., 2022)

Currently, issues remain about widespread acceptance, commercial cultivation, and consumption of the yellow-flesh cassava variants despite their immediate nutritional advantage over white-flesh cassava variants. Some of the challenges have arisen from poor understanding of nutritional benefits of the colored-flesh cassava, misinformation about the nature of development of the variants as genetically modified crops, unwillingness of farmers to change cultivation pattern, and weak governmental commitment to propagation and dissemination for public awareness. These challenges could be stumbling blocks in the sustenance of cultivating yellow-flesh and other colored-flesh cassava (Ayetigbo *et al*., 2018).

2.6 Importance of vitamin A and precursors

Vitamins are organic compounds that are vital for human health, growth, development, reproduction and maintenance, and their deficiencies would pose serious health hazards (Maqbool *et al*., 2018). It is an indispensable micronutrient for humans because it cannot be synthesized in the body but must be obtained from dietary sources (Oruch and Pryme, 2012; Ajibola, 2020).

Vitamin A is a fat-soluble vitamin which can be found in body in three main active forms that are retinol, retinal and retinoic acid, collectively, these compounds are known as retinoids. The cells in the body can convert retinol and retinal to the other active forms of vitamin A as needed. The conversion of retinol to retinal is reversible; whereas the further conversion of retinal to retinoic acid is irreversible. Foods derived from animals provide compounds retinyl esters that are readily digested and absorbed as retinol in the intestine. Foods derived from plants provide carotenoids, some of which have vitamin A. However, other carotenoids, such as lycopene and lutein, are devoid of provitamin A activity (Hailu, 2016) (Table 2.3).

Beta-carotene (β-carotene) is the major plant source of vitamin A precursor that is characterized by two associated retinyl groups (Oruch and Pryme, 2012). The β-carotene content of food varies with the growing conditions and the post-harvest storage of the food. Moreso, the stability of beta-carotene affects its content in foods as Provitamin A carotenoids are easily destroyed by exposure to light, oxygen and during processing, heating and storage (Hailu, 2016). The bioavailability and bioconversion of provitamin A carotenoids can be influenced by various factors such as the digestibility of the food, molecular linkage, amount of carotenoids consumed in a meal, matrix in which the carotenoid is incorporated, intake of dietary fat, type and amount of fiber, alcohol, nutritional status of the individual as well as genetic and host-related factors (Hailu, 2016).

Table 2.3: Carotenoids with vitamin A activity

Compound	Relative Potency
β-Carotene	100
α-Carotene	53
γ-Carotene	43
Cryptoxanthin	57
Lycopene	0
Zeaxanthin	0
Xanthophyll	0

Source: (Hailu, 2016)

The β-carotene is a powerful antioxidant that provide protection against oxidative processes in food systems The antioxidant activity of β-carotene is attributed to their polyene frameworks (Mulindwa *et al*., 2019). Moreover, Antioxidants have proven effective in fighting free radicals, highly unstable compounds that are formed when oxygen combines with certain substances. Free radicals can damage the basic structure of cells and thus lead to chronic diseases such as cardiovascular disorder and cancer and accelerate the aging process. Thus, β-carotene and other carotenoids protect oxidation and free radical damage by quenching singlet oxygen (Hailu, 2016).

Vitamin A and its precursor, pro-vitamin A such as β-carotene, have diverse role and profound effect on heath. Its major roles are promoting vision, participating in protein synthesis and cell differentiation, marinate the health of epithelial tissues and it is known as the anti-infective vitamin, because it is required for normal functioning of the immune system (Whitney and Rady, 2008; Hailu, 2016).

According to Whitney and Rady (2008) the three forms of vitamin A carry out specific functions. Retinal is active in vision and it is also an intermediate in the conversion of retinol to retinoic acid. Retinoic acid acts like a hormone, regulating cell differentiation, growth and embryonic development. Retinol supports reproduction and it is a major transport and storage form of vitamin A.

2.7 Recommended Intake

Generally speaking, genetic background, gender, body size and shape are all important determinants of nutrient requirement (Honi, 2016).

Daily nutritional needs in vitamin A for different class-ages were evaluated by FAO/WHO to tackle vitamin A deficiency. The mean requirement intake is the minimum intake to prevent xerophthalmia in the absence of clinical or sub-clinical infection that expressed as μg retinol equivalents (μg RE). This intake should account for the proportionate bioavailability of performed vitamin A (about 90%) and provitamin A carotenoids from a diet that contains sufficient fat (at least 10g daily) whereas the recommended safe level intake is the average continuing intake of vitamin A to permit adequate growth and other vitamin A dependent functions and to maintain an acceptable total body reserve of the vitamin (WHO/FAO, 2004; Hailu, 2016). Table 2.4 shows the mean requirements and recommended safe intake of vitamin A by each group.

Table 2.4: Estimated mean requirement and safe level of intake for vitamin A, by group (WHO/FAO 2004; Hailu, 2016)

Group	Age	Mean requirements (μg RE/day)	Recommended safe intake (μg RE/day)
Infants and children	0-6 months	180	375
	7-12 months	190	400
	1-3 years	200	400
	4-6 years	200	450
	7-6 years	250	500
Male	11-12	500	1000
	13-15	600	1000
	Adult (15)	600	1000
Adolescents	10-18 years	330-400	600
Adults: Female	19-65 years	270	500
	65+ years	300	600
Male	19-65 years	300	600
	65+ years	300	600
Pregnant women	-	370	800
Lactating women	-	450	850

As the body can derive vitamin A from various retinoids and carotenoids, its contents in foods and its recommendations are expressed as retinol activity equivalents (RAE) (Whitney and Rady, 2008).

2.8 Global Prevalence of Micronutrient Deficiency

Malnutrition (overnutrition, undernutrition, and micronutrient deficiencies) is a physiological state characterized by a deficiency or excess of macronutrients, micronutrients, or both in the human body (Ortiz-Andrellucchi *et al*., 2016).

Micronutrients are an essential nutrient, as a trace mineral or vitamin that is required by an organism in minute amounts that enable the body to produce enzymes, hormones and other substances essential for proper growth and development. Even though they are needed in tiny amounts, consequences of their absence are severe (Hailu, 2016). Micronutrient deficiencies (MNM) are a major public health and socioeconomic concern worldwide. They have an impact on around 2 billion individuals in both developing and developed countries. These are quiet outbreaks of vitamin and mineral shortages affecting people of all ages and genders, as well as specific vulnerable groups (Tulchinsky, 2010; Ajibola 2020).

Micronutrient deficiencies account for about 7.3% of the global burden of disease, with iron and vitamin A deficiencies among the 15 leading causes of the global disease burden (WHO, 2000). It (MNM) is a silent epidemic and is not visible to the naked eye unless and until some adverse effect is observed; therefore, it is also termed as “Hidden Hunger” (Dalbhagat and Mishra, 2021). It is caused by regular intake of low micronutrient content or low bioavailable micronutrient diet (Tam *et al*., 2020). Nigeria loses more than US$1.5 billion annually in Gross Domestic Product (GDP) due to vitamin and mineral deficiencies because many of its staple foods contain very little of these vital micronutrients, according to UNICEF (2004) and the World Bank (2009).

Under-nutrition (53%) causes as much mortality of children younger than 5 years as diarrhea (61%), malaria (57%), pneumonia (52%), and measles (45%) (Black *et al*., 2003; Bryce *et al*., 2005; Black *et al*., 2008; Chadare *et al*., 2019). Up to 3.1–3.5 million of children under 5 years old die every year and women of reproductive age living in low- and middle-income countries because of under-nutrition (fetal growth restriction, suboptimum breastfeeding, stunting, wasting, and deficiencies of vitamin A, iodine, zinc, iron, vitamin D deficiency, rickets, osteomalacia, and

thyroid deficiency) (Black *et al*., 2008; Method and Tulchinsky, 2015; Chadare *et al*., 2019). Black *et al*., (2008) and Bailey *et al*., (2015) opined that women and children are the major targets suffering from consequences of micronutrient deficiency such as poor pregnancy outcomes, children's impaired mental, and physical development.

In addition to the obvious and direct health effects, the existence of micronutrient deficiency has profound implications for economic development and productivity, particularly in terms of the potentially huge public health costs and the loss of human capital formation (Allen *et al*., 2006).

2.9 Vitamin A Deficiency (VAD)

VAD is defined as liver stores below retinol of 20μg/g (0.7μmol/g) (Hailu, 2016). It is considered as one of the most common micronutrient deficiencies worldwide (Stevens, 2015; Hailu, 2016). About 30% of children under 5 years of age are vitamin A deficient (Stevens, 2015). In 2011, a projection of 157,000 children's deaths were connected to VAD worldwide (Black *et al*., 2013). Based on WHO (2011) evaluation, over two billion of global population suffered from insufficiency of micronutrients majorly caused by deficient food intake while that of 2015 (WHO, 2015) evaluation shows that aproximately 250 million preschool children and a substantial proportion of pregnant women are vitamin A deficient with about 250 000 to 500 000 vitamin A-deficient children becoming blind annually, half of them dying within a year of losing their sight. According to Babatunde (2012), children, pregnant women, and people of childbearing age who live in areas where cassava consumption is high are particularly susceptible to vitamin A deficiency.

The main cause of VAD in developing countries is inadequate dietary intake of vitamin A and its precursor carotenoids. The secondary cause of VAD points to the fact that retinol is absorbed from the small intestine dissolved in lipid. In people with a very low-fat intake (less than about 10% of energy from fat), absorption of both retinol and carotene is impaired, and low-fat diets are associated with vitamin A deficiency (Hailu, 2016).

Night blindness is one of the first signs of VAD. In its more severe forms, VAD contributes to blindness by making the cornea very dry, thus damaging the retina and cornea. VAD also contributes to maternal mortality and other poor outcomes of pregnancy and lactation. It also diminishes the ability to fight infections. Even mild, subclinical deficiency can be a problem,

because it may increase children's risk for respiratory and diarrhoeal infections, decrease growth rates, slow bone development and decrease the likelihood of survival from serious illness (WHO, 2023). Vitamin A Deficiency (VAD) and the seriousness of the effects, prevention, and treatment have thereby become a worldwide concern (Okoye and Ezeugwu, 2019).

2.9.1 Combating Vitamin A Deficiency: Techniques

Globally there are short- and long-term strategies to combat micronutrient deficiency that are supplementation, and food-based approaches such as food diversification, fortification (exogenous fortification and biofortification). Food-based approaches promote the consumption of foods that are naturally rich in micronutrient or are enriched foods through fortification (Thompson and Amoroso, 2011; Hailu, 2016).

Nigerian Government under the Ministry of Health implements short term strategy which is the primary prevention strategy which forms part of the routine immunization program, the integrated management of childhood illnesses that is providing vitamin A supplementation to children aged 6-59 months (Ajibola, 2020). Over the years, the coverage of vitamin A supplementation has increased in many low- to middle-income countries including Nigeria; however, the impact of vitamin A supplementation on the reduction of VAD has been at a very slow rate (Afolami *et al*., 2021) According to Honi (2016), such short-term strategy is expensive and not sustainable; moreover, the program does not address women in the post-partum period.

A sustainable strategy to reduce under-nutrition is to increase consumption of foods high in essential micronutrients (Honi, 2016). Sufficient intake of micronutrients at regular basis can cure MNM and also help in boosting immunity and fitness (Maggini *et al*., 2018). The World Health Organization therefore recommends large scale food fortification as a powerful evidence-informed and cost-effective intervention to fight vitamin and mineral deficiencies, including vitamin A deficiency, iodine deficiency disorders, anaemia and iron deficiency, among others. Similarly, the Government of Nigeria has authorized enrichment of some foods with vitamin A ever since year 2000. This has necessitated the development of Pro-Vitamin A Cassava (PVAC), that is planned to support the existing efforts of addressing Vitamin A Deficiency (VAD) by supplying vitamin A via a staple food commonly eaten by the populace (Ajibola, 2020).

According to a recent study by Afolami *et al*., (2021) yellow cassava has been shown to contribute approximately 40 % of the total intake of vitamin A and has the potential to reduce the percentage

of children at risk of inadequate intakes of vitamin A to a low level. Yellow cassava is therefore recommended as a good source of provitamin A in cassava-consuming regions.

2.10 Food Enrichment and Fortification

Food enrichment is the addition of micronutrients lost during processing to a food product, whereas fortification is the addition of additional micronutrients that were not present (or were present in limited amounts) previous to processing (Nwadi *et al*., 2020). Most times however, some researchers and health organizations make use of both terms interchangeably. For instance, the World Health Organization (WHO) defines fortification as the intentional addition of one or more microelements to food products in order to increase their content or prevent particular deficits and to provide health benefits (Allen *et al*. 2006). In addition, it is possible to enrich the product with active substances, not naturally found in a certain type of food (WHO 2015). Fortified products can be classified as functional foods only if the content of the added ingredient is high enough to trigger/cause a clinically documented health effect (e.g. reduce the risk of disease) (Poniedziałek *et al*., 2020).

Fortification can lead to rapid improvements in the micronutrient status of a population at a reasonable cost, and the foods to be fortified must be consumed adequately by a large proportion of the target individuals in a population (Oyeyinka and Oyeyinka, 2016). It is considered as the most appropriate preventive approach against malnutrition caused by micronutrient deficiencies (Bhagwat *et al*., 2014). For many years, it has been used as a cost-effective means to prevent micronutrient malnutrition (Method and Tulchinsky, 2015). Considerable studies have been carried out to develop food fortification in developing countries (Honi 2016; Okoye and Ezeugwu, 2019; Ajibola, 2020). The success of this fortification allowed many countries to effectively combat micronutrient deficiencies in populations. It has a much wider and more sustained impact on providing essential micronutrients to targeted populations (FAO, 2006) and many fortification programs run in both developed and developing countries (Bailey *et al*., 2015). In some countries, it has become mandatory and/or going to become mandatory to add micronutrients in certain staple foods (Chadare *et al*., 2019; McAuliffe *et al*., 2020). Millions of Nigerians regardless of ages, genders and geographical locations obtain inadequate vitamin A (Aniedu and Omodamiro, 2012) despite the fact that the government of Nigeria has authorized enrichment of some foods with vitamin A ever since 2000. The support of the existing efforts to address Vitamin A deficiency via

a staple food commonly eaten by the populace has necessitated the development of Pro-vitamin A cassava (PVAC) (Ajibola, 2020).

Food fortification can take several forms, and different techniques and procedures can be used (Liyanage and Hettiarachchi, 2011). Depending on the target group, fortification can take many forms. Mass fortification consists of enriching food that is commonly eaten (such as cereals, condiments, and milk) by the general population and can be considered a public health strategy to increase intake of critical nutrients. Targeted fortification is, in turn, used for products intended for a specific group, e.g. complementary foods for infants or vegan foods enriched with vitamin B12. This type of fortification is obligatory or voluntary, depending on the public health significance of the problem it is seeking to address. Market-driven fortification (sometimes called open-market fortification) is aimed at increasing profits and the prestige of producers and is always voluntary, but is governed by regulatory limits (Poniedziałek *et al*., 2020) .

More and more, the aim of food fortification is to improve people's health instead of deficiencies' prevention (Dwyer *et al*., 2015; Chadare *et al*., 2019). And elimination of these deficiencies is essential, not only to improve health, but also for sustained economic growth and national development (Mishra, 2011). Thus, to overcome all these challenges, development of nutritious and cheap foods from locally available foods is important. Efforts are therefore being made by employing food-to-food fortification (Onuoha and Ene-Obong, 2005).

2.10.1 Food-to-food fortification

A novel approach in managing micronutrients using the food-based strategy is the food-to-food fortification (FtFF). The FtFF is defined as a strategy of adding one or more than one micronutrient-dense food to a recipe or the replacement of nutrient deficient/antinutrient rich ingredient, to effectively enhance the quantity of bioavailable micronutrient/s, in view of satisfying the population with low intake of bioavailable micronutrients (Kruger *et al*., 2020). It is the approach to add micro-or macro-nutrient dense food into nutrient deficient foods for increasing its nutritional properties (Kruger *et al*., 2020; Vishwakarma *et al*., 2021). This approach helps to improve the overall micronutrient level of staple or regular foods (Chadare *et al*., 2019).

Thus, for combating micronutrient deficiency and even improving public health, locally available foods should be added as natural fortificants in the staple food and processed foods. The FtFF in

processed foods like snack, pasta, biscuit, cookie, bread, etc. can provide multiple nutritional and health benefits and can contribute to fighting against MNM (Vishwakarma *et al*., 2022). These foods must be rich in one or more than one type of micronutrient and may include the advantage of providing some additional non-targeted properties such as protein, dietary fiber, and antioxidants (Vishwakarma *et al*., 2022).

For the FtFF, the prominent source of natural fortificants is mostly plant-based food due to safety and ease in availability among the target population (Chadare *et al*., 2019). The FtFF follows the process of adding nutrient rich food in the staple diet in achieving target levels of one or more than one type of micro-and/or macro nutrient (Vishwakarma *et al*., 2022). In practice, a micronutrient-rich food known as fortifying food is first selected and then blended with a common staple food to provide the required micronutrient levels with minimum changes in consumer acceptability (Chadare *et al*., 2019). The staple diet can be local foods and/or processed foods like snack, pasta, biscuit, bread, cookies, etc (Vishwakarma *et al*., 2022). In most cases, it is preferable to use food vehicles that are centrally processed, and to have the support of the food industry for an effective impact of food-to-food fortification. For this technique, the rate of fortification varies considerably (1%–50%) and depends on the compatibility of the vehicle (the staple food) and the fortificant (Chadare *et al.*, 2019).

For both classical food fortification and food-to-food fortification, the main objective is to improve the nutritional quality of the fortified food without losing sight of the acceptability criteria (mainly the food organoleptic quality). As such, next to the laboratory formulation of the fortified food, a sensorial evaluation is necessary to assess acceptability levels (Chadare *et al.*, 2019).

More investigation however needs to be done on applying FtFF on ready-to-eat, ready-to-cook, and ready-to-serve food segments like chocolates, ice-cream, noodles, pasta, pizza, burger, flakes, health drinks, and other fast foods. Because these segments are quite deficient in micronutrients and are being consumed on a frequent basis in today's lifestyle (Vishwakarma *et al*., 2022).

2.10.2 Effect of Food-to-Food Fortication on the food quality

The raw ingredients used in Food-to-Food Fortification improve the micronutrient content and affect other characteristics of the developed product (Vishwakarma *et al*., 2022).

2.10.2.1 Colour

Colour is an important property of a food product that decides the preference of the consumers. The colour of a product generally depends on the ingredients used in the food formulation (Dalbhagat *et al*., 2019; Dhull *et al*., 2020). Most times if not all times in FtFF, the addition of fortificants tends to affect greatly the colour of the fortified food product (Srivastava *et al*., 2010; Pratama *et al*., 2018; Ningsih *et al*., 2020)

In addition to ingredients, the process operations also affect the color of the product. For instance, during baking, Maillard reactions occur via reactions between reducing sugar and amino acids which result in the darkening/browning of the food product (Nwadi *et al*., 2020).

2.10.2.2 Sensory attributes

Ingredients play a key role in enhancing the quality of the product in terms of appearance, color, texture, taste, and overall acceptability. The higher level of addition of natural fortificants is decided by sensory evaluation (Shori *et al*., 2020). Most of the time, the compromise over the nutritional enrichment has to be done due to adverse effects on the sensory attributes (Vishwakarma *et al*., 2022).

2.11 Composite Flours

Composite flour is a cutting-edge flour that has received a lot of attention in both research and the production of food products (Hasmadi *et al.*, 2020). Composite flour is defined as a mixture of flours obtained from roots and tubers rich in starch such as cassava, yam, potato, and protein-rich flour (including groundnut, soybean etc) and cereals (including maize, millet etc), with or without wheat flour developed to meet specific functional requirements and nutrient composition (Noorfarahzilah *et al*., 2014). This definition indicates that adding wheat flour to composite flour is optional.

A number of factors, such as the detrimental economic effects of wheat importation on low or non-wheat producing countries, poor protein quality due to lysine deficiency, and the association between gluten sensitivity and celiac disease have made it necessary to substitute nutrient-dense locally grown crops, such as root and tubers, grains, legumes, and so on, in or for wheat (Arise *et al*., 2021).

At the moment, attempts are being made to identify the non-wheat bases that might be used in most developing countries to replace wheat in the production of baked goods and other convenience foods. Because it encourages the production of local crops and decreases the importation of wheat flour, this is thought to be advantageous for developing nations (Hugo *et al*., 2000; Hasmadi *et al.*, 2020). More specifically, some of the advantages of composite flour in the food sectors worldwide, particularly in a developing country like Nigeria, include progress toward optimum yield of native species, provision of nutrients, farming, generation of income, and assistance with rural development by creating employment opportunities and thereby improving local economies (Ajibola, 2020). Compared to flour milled from a single crop, composite flour provides more nutritional value in terms of minerals, vitamins, fibers, and proteins. According to Shanti *et al*., (2005), the composite flour mixture could offer a nutritionally balanced diet.

2.12 Cookie

Cookies are small, flat, sweet, baked goods, usually containing flour, egg, sugar, and either butter, cooking oil or fat (Abayomi *et al*., 2013). They are nutritious ready-to-eat baked snacks available in different shapes and sizes at an affordable cost (Vijerathna *et al*., 2019).

They are one of the most popular baked goods consumed as snacks among all age groups in many countries due to their creamy taste, unique texture and low water activity which defines their long shelf life (Petrovic *et al*. 2016; Ubbor *et al*., 2022). Compared to biscuits, cookies tend to be larger with a softer chewier texture (Chinma *et al*., 2012).

Conventionally, cookies are produced from wheat flour due to its gluten content which improves its texture (Oluwafemi and Seidu, 2017). However, several studies have reported the use of composite flour in cookies production (Akusu *et al*., 2019; Obinna-Echem and Robinson, 2019; Ukeyima *et al*., 2019; Bello *et al*., 2020; Ubbor *et al*., 2022).

Okpala and Okoli, (2011) reported that cocoyam can be used in the production of cookies. Apotiola and Fashakin, (2013) also reported the production of cookies by replacing wheat with a certain percentage of yam flour and soybean which makes it possible for other root and tubers and legumes to be used in the production of cookies. All these efforts are aimed at reducing dependence of bakeries on wheat flour, improving the nutritional values of cookies and finally, to add value to indigenous crops.

2.12.1 Ingredients in Cookie production

Cookie production (fig. 2.3) involves the use of different ingredients which are usually active for the specific attribute they possessed and/or result they have on the finished product. They include;

Flour: Flour is the main ingredient and bonding agent of cookie, representing about 60% to 70% of a typical formulation which gives a framework around other ingredients grouped in varying proportions. Excessive hardness of flour may cause difficulties in spread-ability for cookies.

Leavening agent: It controls spread-ability, size and volume of the dough; gives acceptable crust colour by regulating the acidity or alkalinity of dough.

Salt: Salt at 1-2 % improves taste, it has a stabilising effect on yeast fermentation, prevents contamination and toughening effect on gluten, it retards proteolytic activity.

Sugar: Many large-scale bakeries add either sucrose or glucose (about 2% to 3%) as an additional source of readily fermentable sugars. They also supply flavour and, when the dough is baked, colour.

Fat/Shortening: The addition of 0.1% to 0.2% fat gives cookie a special taste. Vegetable oils and compounds of vegetable oil increases the volume, gives a thinner and tenderer cookie. It also increases the calorific or energy value of the cookie. Furthermore, fat acts as lubricants especially during kneading. However, excessive use of fat could lead to loss of volume.

Flavouring Ingredient: It gives sweet aroma to the cookie.

Egg: Eggs can be used as an agent to soften cookie during baking. The fat contained in egg yolk could help to tenderize the cookie. Furthermore, albumen (egg white) acts as structure builders as proteins congeal while baking. Whole eggs supply combined characteristics of shortness, aeration, and softness in baked products.

Milk solids: Milk solids exert slight binding action on dough, promotes nourished assessment and milk sugar lactose which enhances crust colour of the cookies.

Miscellaneous Flavours: Vanilla flavour, Oatmeal, peanuts, spices, chocolate chips and nuts serve as flavour which can be used to produce specific flavours.

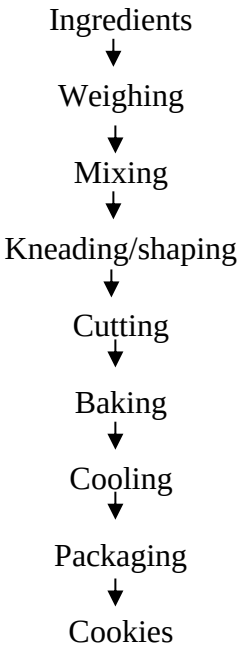

Figure 2.3: Flow chart for production of cookie (Abayomi *et al*., 2013)

2.13 Baking

Baking is an excellent process technology for producing Ready-to-Eat food items like biscuits, cookies, and bread. Various baked items has been fortified using natural fortificants (Okoye and Ezeugwu, 2019; Candriasih *et al*., 2019; Olatunde *et al*., 2016). The high temperatures tend to trigger a release of protein-bound minerals like calcium, magnesium, zinc, and iron via softening the food matrix. This may result in improvement in the nutritional value of the baked product (Agrahar-Murugkar, 2020). It has also shown to be able to enhance shelf life (Akbar *et al*., 2020).

2.14 Packaging

Food packaging has a crucial function in modern food industry, since it contributes to preserve food products quality and guarantee food safety during its shelf-life (Ghaani *et al*., 2016). Traditional food packaging has four basic functions: protection and preservation, containment, communication and marketing, and convenience (Biji *et al*., 2015). Packages are used to protect the product from spoilage and damage brought about by environmental factors, such as microbes, insects, light, heat, oxygen, water vapor, smells, dirt, dust, etc. (Salgado *et al*., 2021). They can take different shapes and sizes to hold food products, with the aim of improving logistical efficiency (Yam and Lee, 2012). They communicate with the consumer by means of written texts (the ingredients list, nutritional facts, preparation instructions, etc.) and the brand logo. They adapt to consumer's lifestyle, for example, saving time (suitable for ready-to-eat meals) or facilitating their handling (easy to open, reclosable or suitable for microwave) (Vanderroost *et al*., 2014; Salgado *et al*., 2021). In addition, Packaging plays a substantial part in predicting food storage stability (Ajibola, 2020). Moreover, Snack food packaging has become a critical component of the sales process, significantly impacting consumers' buying decisions (Spruit and Almenar, 2021; Wang *et al*., 2023). Correct choice of food packaging material is therefore essential (Ajibola, 2020).

Some of the common food packaging materials adopted in most food industries comprises glass, metals, paper and paperboards and plastic containers (Gulia *et al*., 2014). Polyethylene and polypropylene packaging materials have an effective mixture of properties comprising plasticity, strength, weightlessness, steadiness, moisture and chemical resistance and simple processing. They have the advantage of being reusable and recyclable (Marsh and Bugush, 2007; Ajibola, 2020). Hence, polyethylene packaging material was considered for this novel composite cookie.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Yellow root cassava [IBA48100 (Security)] (Plate 3.1) was sourced from National Root Crops Research Institute (NRCRI), Umudike, Umuahia, Abia State. Dried Bambara groundnut seeds (cream coloured variety) (Plate 3.2), and other ingredients required for cookie production were procured from Bodija, a local market in Ibadan, Oyo State, Nigeria. Analytical-grade supplies were used for all other equipment and chemicals.

3.2 Methods

3.2.1 Research design

This study employed a Completely Randomized Design (CRD), with several formulations serving as the treatment.

3.2.2 Processing of yellow root cassava into flour

Yellow root cassava was processed into flour using a slight improvement on the procedure outlined by Okoye and Ezeugwu, (2019). Wholesome cassava roots were washed (with clean water) to remove adhering soil and then hand peeled with sharp stainless steel kitchen knives. The peeled roots were rewashed (with clean water) and rinsed, a commercial hammer mill was used to grate yellow root cassava into a mash and bagged to remove water. The cake that resulted from the dewatering process was broken up into small pieces and sieved. The sifted semi dried mash was oven dried (hot air electric drying oven) at 50^0C for 4 hours. The dried samples were then milled and sieved into resultant flour through a 250 mm mesh sieve to obtain smooth flour (yellow-root cassava flour) with a uniform particle size. The flour was then packaged in a black polypropylene bag and kept at ambient temperature until further needed. Figure 3.1 depicts the unit operations used to turn yellow root cassava into flour.

Plate 3.1: Pro-vitamin A cassava variety [IBA48100 (Security)]

Plate 3.2: Bambara groundnut

3.2.3 Preparation of Bambara Groundnut Flour

Bambara groundnut flour was be prepared with a slight modification of the method described by Arise *et al*., (2022). Bambara groundnuts was sorted to remove extraneous materials such as stone and dirt and to separate insect-infested seeds from desirable ones. Broken, wrinkles and immature seeds were also sorted out and soaked in water for 4 days at room temperature in a bucket with lid. After soaking, the seeds were then dehulled (the soaking eased removal of the outer coat) and dried in a cabinet oven at 50°C for 2 days. The dehulled grains were thereafter ground in a Warring laboratory mill blender (HGBTWTS3, Torrington, CT, USA) and sieved through a screen mesh of 250 mm to obtain fine flour. The flour was subsequently kept in air-tight ziplock bags prior to use; Figure 3.2.

3.2.4 Formulation of Composite Flour Blends (Flour formulations for cookie production)

In order to produce the experimental composite flour, a substitution procedure was used (Table 3.1).

The flours were thoroughly mixed using Eurosonic electric blender for 1min to obtain homogenous flour blends. The samples were tagged and kept in airtight containers at ambient temperature (30 ± 2°C) until required.

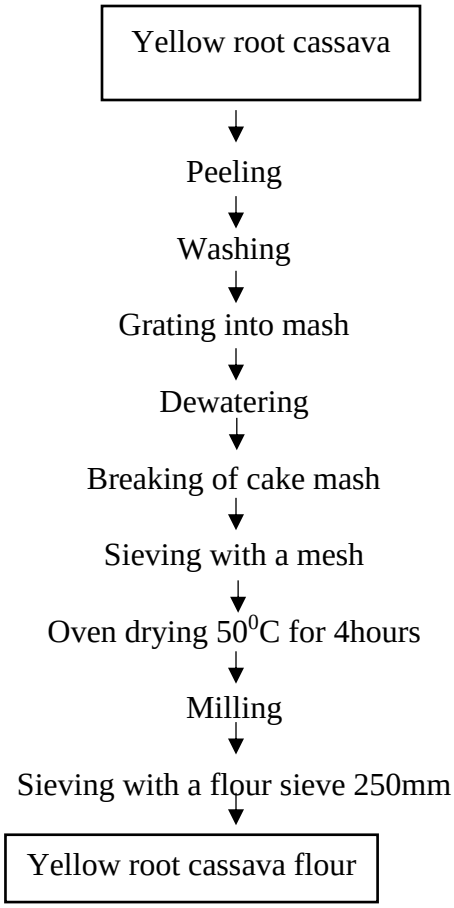

Figure 3.1: Production of flour from yellow root cassava (Okoye and Ezeugwu, 2019).

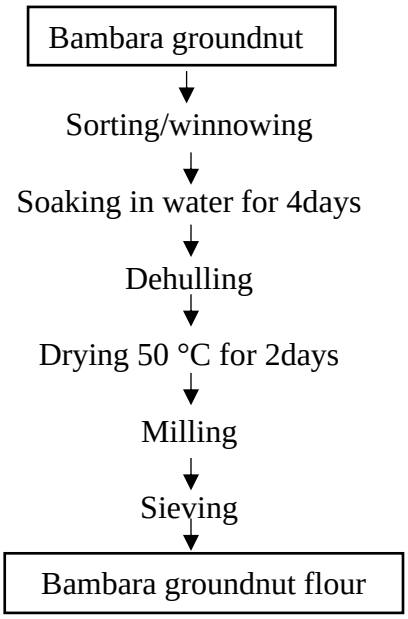

Figure 3.2: Production of flour from Bambara groundnut (Arise *et al*., 2022)

Table 3.1: Formulation of Pro-vitamin A Cassava (PVAC) and Bambara groundnut (BN) composite flours for cookie production

Formulation	Sample code	% of ingredient in the formulation	
		PVAC	BG
PVAC_ (0%)	A	100	0
PVAC_ (20%)	B	80	20
PVAC_ (40%)	C	60	40
PVAC_ (60%)	D	40	60
PVAC_ (80%)	E	20	80
BG_ (100)	F	0	100

PVAC=Provitamin Cassava; BG = Bambara Groundnut

3.2.5 Determination of the Functional Properties of Flour Blends

3.2.5.1 Water and Oil Absorption Capacity

The procedure for the determination of water and oil absorption capacity was adopted from Adewumi *et al*., (2022). Sample (1 g) was mixed with 10mL distilled water or vegetable oil in separate pre-weighed 50 mL centrifuge tubes. After being vortexed for 1 minute, the sample was held at room temperature for 30 minutes, and then separated by centrifuging at 4000 × g for 30 minutes. The supernatant was decanted and surplus water or oil in the upper phase was drained for 15 minutes. The tube containing the residue was reweighed in order to calculate the amount of water or oil retained per gram of the sample. Water and Oil absorption capacity (WAC) was calculated using Equation 3.1 and 3.2 respectively.

$$WAC\ (g/g)\ =\ W2 -\ W1/W \text{-------3.1}$$

$$OAC\ (g/g)\ =\ W2 -\ W1/W \text{-----3.2}$$

where, W = weight of dry sample, W_1 = weight of the tube plus the dry sample, and W_2 = weight of tube plus wet sample.

3.2.5.2 Bulk density

Bulk density was determined by the procedure described by Eltayeb *et al*., (2011). Samples were placed in a 25 ml graduated cylinder and packed by gently tapping the cylinder on the bench top 10 times from a height of 5 cm on a laboratory bench until no more sample could be absorbed and the volume of the sample was recorded. The procedure was repeated two times for each sample and the bulk density was computed as g/ml of the sample.

$$Bulk\ density\ =\ Weight\ of\ sample\ (g)\ /Volume\ of\ sample\ (ml) \text{ ----3.3}$$

3.2.5.3 Dispersibility

The technique proposed by Awoyale *et al*., (2015) was followed. Samples were weighed (10 g each) into 100 ml measuring cylinder and distilled water added to reach a volume of 100 ml. The set up was stirred vigorously and allowed to settle for 3 h. The volume of settled particles was recorded and subtracted from 100. The difference was then reported as percentage dispersibility.

3.2.5.4 Swelling and solubility index

The method proposed by Ajibola, (2020) was used. A gram of flour blend was poured into known weight of centrifuge container. Fifteen millimetres of purified water was added and gently stirred at a low speed for about 5 minutes. The mixture was boiled in thermostatic water bath at 80^0C for 40 min with constant stirring to prevent formation of lumps. This was allowed to cool before being separated in a separator for 20 minutes at 2,200 rpm. The fluid above the sediment was transferred instantly into a previously weighed container and oven dried at about 100^0C till a constant mass was attained. This was cooled and weighed. Mass of residue was weighed and noted.

$$Swelling\ power\ (\%) = Mass\ of\ residue/\ mass\ of\ sample \times 100 \quad \ldots 3.4$$

$$Solubility\ index\ (\%) = Weight\ of\ soluble/Sample\ weight \times 100 \quad \ldots . 3.5$$

3.2.5.5 Foaming capacity

This was determined using the method described by Folaju *et al*., (2018). One gramme of flour sample was dispersed in distilled de-ionized water. The dispersed sample was whipped in with the aid of electric blending machine using a Silver Crest Blender at 1600 rpm. The sample was whipped continuously for 8 minutes at ambient temperature. The foam volume was measured in 250ml measuring cylinder after the whipped sample was poured into it. The foam volume was expressed as a percentage of the volume occupied by the sample prior to whipping. The foaming capacity (FC) was calculated as:

$$FC\ (\%) = Volume\ of\ foam\ after\ whipping - Volume\ of\ foam\ before\ whipping/Volume\ of\ foam\ before\ whipping \times 100$$

3.2.5.6 Emulsion capacity

The emulsion capacity (EC) of the flours is determined according to the method of Kacou *et al*., (2018). Two (2) g of flours (ME) are dispersed in 50 ml of distilled water contained in an Erlenmeyer flask. The mixture is homogenized with a magnetic stirrer for 20 minutes. The suspension is transferred to a centrifuge tube and then ten (10) ml of oil (V0, density 0.88 g/ ml) are added thereto. This mixture is stirred continuously for 5 minutes and then heated in a water bath for 15 minutes at 85 ° C. The tube is removed, then cooled to room temperature (25 ° C) for

10 min and centrifuged at 4500 rpm until the volume of oil (V1) separated from the emulsion (V2) becomes constant. The results are expressed as a percentage of emulsified oil per gram of flour used.

$$EC\ \% = V2/ME\ x\ 100$$

3.2.5.7 Least Gelation Concentration

The method of Awoyale *et al*., (2015) was used in the determination of the least gelation concentration (LGC). Appropriate sample suspensions were weighed into 5 ml distilled water each to make 2–20 % (w/v) suspension. The test tubes containing these suspensions were heated for 1 h in boiling water (bath) followed by rapid cooling under running tap water. The test tubes were further cooled for an hour under running water and the LGC was determined as the concentration when the sample from the inverted test tube did not fall or slip.

3.2.6 Proximate Composition of Flour blends

Moisture, crude protein, fat, ash, and crude fibre of samples were determined using the standard methods described in the AOAC (2012). The carbohydrate content was determined as the weight difference using moisture, crude protein, lipid, crude fibre, and ash content data while the energy value (kcal/100 g) was evaluated by using the Atwater conversion factor (9 × % lipids + 4 × % proteins + 4 × % carbohydrates) (Kobue-lekalake *et al*., 2022).

3.2.6.1. Moisture Content Analysis

The moisture content was determined by weighing out 2g of each of the samples into a dry pan of a known mass, charged into the oven at a temperature of 105^0C for 3hrs. The dried samples were then withdrawn from the oven and placed in a desiccator to cool. They were weighed using the analytical balance (electronic) and the whole process was repeated until a constant mass was obtained. The difference in mass was used to calculate the percentage (%) moisture content as follows;

$$\%\ moisture - \left[\frac{M2 - M3}{M2 - M1}\right] x\ 100$$

Where M_1 – mass of dish

M_2 – mass of dish + sample before drying

M_3 – mass of dish + sample after drying

3.2.6.2 Crude Fat Analysis

A Soxhlet extraction unit with a reflux condenser and a small round bottom flask (250ml) was used. The flask was weighed after washing and drying and half filled with light petroleum ether (Boiling point 40-60^{0}C) and fitted back to the unit. Two (2) gram portion of each sample was wrapped with Whatman filter papers and gradually lowered into a thimble then fitted into the reflux flask. The boiling flask containing 200ml petroleum ether was heated with heating mantle for 5h. The ether in the bottom flask, evaporated, condensed in the reflux flask until it filled. Then it was refluxed into the boiling flask carrying the extracted fat and oil. Heating was stopped after 5hrs and the bottom flask with solvent was allowed to cool. The ether in the flask was evaporated at 60^{0}C and its oil content dried. The flask with the oil was reweighed. The difference was used to calculate the percentage (%) of crude fat in the sample.

$$\% \, Crude \, fat - \, [Mass \, of \, fat / Mass \, of \, sample] \, x \, 100$$

3.2.6.3 Crude Protein Analysis

The Kjeldahl method was adopted. Half a gram (0.5) of each dry sample was weighed and placed in a Kjeldahl digestion flask. A blank experiment was also setup involving the digestion of all material except the sample. One table spoon of selenium catalyst was added into each of the flask and mixed with 10ml of concentrated H_2S0_4.The mixture was heated to red hot temperature under a fumed cupboard for 2hours until a clear solution was obtained. The clear solution (the digest) was transferred quantitatively to 100ml volumetric flask and diluted to mark with distilled water. The digest was mixed with equal volume of 45% NaOH solution in a semi micro kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into 10ml of 40% boric acid solution containing about three (3) drops of mixed indicator, methylene red and bromocresol green. A total of 50ml distillate was collected and titrated against 0.02N H_2S0_4 solution. The above distillation process was also carried on the blank sample. The titer value was subtracted from that of the sample and the difference used to calculate the crude protein as thus;

The % of nitrogen content is given by;

$$\%N = \, (14 \, x \, Na \, (VF/VA) \, (100/w) \, XT$$

Where T-titer less blank

Na-normality of acid used

VF – total volume of aliquot

VA – Aliquot volume distilled

W – Mass of sample analyzed

Thus;

$$\%\ crude\ protein\ (\%P) = \quad \%N\ x\ 6.25$$

3.2.6.4 Crude Fibre Analysis

Two grams (2g) of defatted sample was boiled in 200ml of 125% H_2S0_4 for 30 minutes. The boiled sample was washed with hot water using a two-fold muslin cloth to retain particles. The retained particles were returned to the flask and boiled again in 200ml of 125% NaOH solution and was again washed with hot water and allowed to cool before being transferred to a weighed porcelain crucible. The sample was transferred to the oven to dry at 105^0C to a constant weight and was subsequently placed in muffle furnace at 550^0C for 4h and finally cooled in a desiccator and reweighed. By differences in mass, the mass of the fibre was then determined and used in calculating the percentage (%) fibre:-

$$\%\ crude\ fibre\ = [(loss\ in\ mass\ on\ ignition\ x\ 100)]/Mass\ of\ sample$$

3.2.6.5 Ash Content Analysis

A measured mass (2g) of sample was weighed into a crucible of known weight. The sample in the crucible was placed in a muffle furnace at a temperature of 550^0C for 3h until samples were free of carbon. The crucible was cooled in a desiccator to room temperature and weighed. The percentage (%) ash was calculated as thus;

$$\%Ash\ = \ Mass\ of\ ash\ x\ 100/Mass\ of\ sample$$

3.2.6.6 Carbohydrate Content and Energy value

Minerals and vitamins occur in minute quantity. Thus, to determine the % carbohydrate content, the sum of the contents, moisture (%M.C), ash (%A), protein (%N), fat (%F) and crude fibre (%C) has been deducted from the total mass and is given by;

$$\%\ carbohydrate = \ 100 - (\%MC + \%A + \%N + \%F + \%C)$$

$$Energy\ value\ (Kcal/100\ g)\ =\ 4\ x\ protein\ (\%)\ +\ 9\ x\ fat(\%)\ +\ 4\ x\ carbohydrate\ (\%)$$

3.2.7 Mineral Analysis on flour blends

The calcium, sodium, magnesium, phosphorus, potassium, iron and zinc contents of the flour blends were determined using the dry ashing method (AOAC, 2012). The sample (2 g) was ashed in a furnace at 550°C for 18 hrs (the ash gotten from ash analysis was rather used). The ash was dissolved in 10 ml of 1.0 M HCL, filtered into a 100 ml volumetric flask and made up to mark with distilled water. This was then used to determine the mineral concentration in Atomic absorption spectrophotometer (Jenway 7305, Bibby Scientific, London, UK) using prepared standard of the different mineral elements to be analyzed (Ca, Na, K, Mg, P and Fe). The results were expressed in g/100g.

3.2.8 Determination of beta carotene on flour blends

Pro-vitamin A was determined using a slight modification of the method described by Honi, (2016) and Fortunatus *et al*., (2021). About 2-3 g of flour was weighed, transferred into a mortar and was grounded with 50 ml of acetone (acetone refrigerated at 40^0C for 2 hours prior to use) being added slowly then filtered using cotton wool plugged into the fume. The extraction was repeated until the sample from the mortar was devoid of colour. About 40 ml of petroleum ether was put in a separating funnel (250 – 500 ml capacity) and acetone was added. Distilled water was added slowly along the neck without shaking to avoid emulsion formation and when emulsion occurred, saturated sodium chloride (NaCl) solution was added to prevent formation of an emulsion. The two phases were then left to separate and the lower aqueous layer discarded, the sample was washed 3-4 times with distilled water (approx 200 ml) each time to remove residual acetone, in the last phase washing was done ensuring no any amount of the upper phase was discarded. Then the upper layer was collected into 50 ml flask using 15g anhydrous sodium sulphate filter arrangement to remove residual water. The volumes of carotenoid solution were then adjusted to 50ml with petroleum ether, and absorbance readings were taken using a UV-VIS Spectrophotometer. The absorbance was determined at a wavelength of 450 nm and beta carotene was calculated as follows;

$$Total\ Carotenoid\ \left(\frac{\mu}{g}\right) =\ A\ \times volume\ (ml) \times\ 10E4\ /\ A1\%1cm\ \times\ P\ (g)$$

Where A = absorbance,

Volume = total volume of extract (50ml),

$A^{1\%}1cm$ = absorption coefficient of β-carotene in Petroleum ether (2592)

P(g) = Sample weight

3.2.9 Determination of antinutritional factors in flour (main ingredients)

The procedure outlined by Ajibola and Olapade, (2021) was followed to determine the hydrogen cyanide, tannin and phytate contents of the flours.

3.2.9.1 Determination of hydrogen cyanide content

Fifteen grams of flour was measured and standardised with 125 millimetres of 0.1 M orthophosphoric acid. The homogenate was separated by removing the liquid above sediment. From extract, 0.1 ml was taken and treated with linamarin stock to obtain entire cyanogenic potential. Additional test was performed using 0.1 ml of the extract nevertheless 0.1 ml of 0.1M phosphate buffer (pH 6.0) was utilised to provide non-glucosidic cyanogenic potential. The third investigation was performed using 0.6 ml of the extract, this was poured into 3.4 ml of McIlvaine buffer (pH 4.5). The free cyanogen was obtained by appropriate agitation of the mixture using 0.2 ml of 0.5% chloramin T and 0.8 ml of colour component. A normal arc was achieved by plotting absorbance values in vertical-axis and normal concentration values in horizontal-axis: linamarin = 125 ml/(0.01093 x sample weight); Non-glucosidic cyanogen = 125 ml/(0.03176 x sample weight); permitted cyanide = 125 ml/(0.04151 x sample weight).

3.2.9.2 Determination of tannin content

The modification includes the use of 99% methanol instead of 70% acetone. Five millimeters of 1% HCl in methanol was poured into 0.5 g of flour. Combination was shaken to agitate uniformly for 2 min with the use of vortex mixer and centrifuged at 3,000 revolution per minute for ten minutes.

A millimetre of liquid above the sediment was stirred with 0.5 ml Folin Ciocalteau reagent. Similarly, 0.5 millimetre of 20% sodium carbonate and eight millimetres of purified water was poured to the combination. Minimum of thirty minutes was permitted to change colour and

absorbance was noted at 760 nm by means of Genesys UV-visible spectrophotometer of model 10S. Curve of tannic acid in blank and standard was plotted alongside absorbance. The tannin composition was estimated as mg tannic acid equivalent from a line regression equation obtained from a standardisation curve. The sample tannin content was then read from the curve.

3.2.9.3 Determination of phytate content

A gram of each sample was dissolved in 25 ml of trichloroacetic acid (TCA) and gently shook in an orbital shaker for 3 h. The extract was removed by centrifuging at 2000 rpm for 15 min. Ten millimetres of liquid above the sediment was poured into quantifying tube and 4 ml of iron III chloride was added. This was boiled in water bath for 45 min, centrifuged and poured out. The residue was cleaned twice by dissolving in 25 ml of 3% TCA. This was heated in water bath for 10 min, centrifuged and the residue cleaned with purified water. This was dissolved in 5 ml of purified water, 2 ml of 1.5 N NaoH was added and mixed. The volume was increased to 30 ml with purified water.

The combination was boiled in water bath for 30 min, filtered hot into 100 ml container and the residue was dissolved with 40 ml of hot 3.2 N trioxonitrate acid. The filter paper was cleaned with water, cooled to room temperature and diluted to the mark. From the aliquot, 0.5 ml was weighed into another 100 ml flask, diluted to 70 ml with water. The absorbance of this was read at 470 nm of Genesys UV-visible spectrophotometer of model 10S. The phytate content was obtained from a line regression equation obtained from a standardisation curve. The sample phytate was then read from the curve.

3.2.10 Determination of Colour of flour (main ingredients)

The colour of flours were determined using Konica Minolta Chroma metre of model CR-410 (Japan). This apparatus was standardised using a white tile. The results were expressed in accordance with the CIELAB system where:

L (L = 0 [black], L = 100[white]),

a (-a = greenness, +a = redness),

b* (-b = blueness, +b = yellowness)

The three dissimilar colour attributes: *L**, *a** and *b** were noted. The *L** determines brightness of which the highest value denote lighter, while *a** determines the equilibrium between redness and greenness of samples that displayed in positive and negative numbers respectively. The *b** designates the equilibrium between yellowness (+) and blueness (-). As *a** and *b** values nearer to zero, it shows reduced concentrated colour, while values far away from zero correspond to more concentrated colour.

The samples were evaluated three times (n=3) by placing the measuring head in an haphazard manner to diverse positions on top of the sample (Ajibola, 2020).

3.2.11 Cookie Preparation

A slight adjustment of the procedure described by Arise *et al*., (2021) was followed for cookie production. Specifically, flour, 100.0 g; hydrogenated vegetable fat, 40.0 g; granulated cane sugar, 25.0 g; fresh whole egg, 31.0 g; full-fat filled powdered milk, 7.8 g; nutmeg, 0.3 g; liquid vanilla flavour, 5.0 mL; salt, 1.0 g; and baking powder, 1.0 g were used. Fat and sugar were creamed using a hand mixer at medium speed for 5 min. Eggs and vanilla flavour were added while mixing and further mixed for 30 min. Dried ingredients including; milk, nutmeg, flour, baking powder, and salt were weighed out separately, mixed thoroughly and then added to the cream mixture followed by another mixing for 10 min to form a dough. The dough was sheeted to a uniform thickness of 0.25 cm and cut into circular shapes of 4.5 cm diameter each. The shaped dough was baked at 185°C for 20 mins. The cookies, after being allowed to cool at room temperature, were packaged in a transparent polyethylene bag and kept at refrigeration temperature of 4°C until required.

Six samples of cookie for each formulation were obtained and were coded accordingly based on the flour blend for which they were produced as Ac, Bc, Cc, Dc, Ec, and Fc. The process was replicated at least in triplicate to obtain enough samples to analyze the different parameters in this study.

3.2.12 Determination of physical properties of cookie samples

The physical properties of the cookies were determined using the methods described by Arise *et al.*, (2021) and Offia-Olua and Akubuo, (2019).

3.2.12.1 Weight

Weights of the cookies were taken on an electronic weighing balance.

3.2.12.2 Diameter

The diameter was assessed using a digital vernier caliper.

3.2.12.3 Thickness

The thickness was also assessed using a digital vernier caliper

3.2.12.4 Spread Ratio

The spread ratio of each cookie was expressed as the diameter of such cookie divided by its thickness.

3.2.12.5 Breaking Strength

Break strength tool was used. Cookies were placed on each other between two parallel metal bars maintaining 3cm away. Weights were placed on cookies till they were cracked but application of least weight caused breaking of cookies and was noted as the breaking strength.

3.2.13 Colour of cookie samples

Colour of cookie samples were investigated with the use of Konica Minolta Chroma metre of (model CR-410, Japan) as described by Ajibola, (2020) as explained in section 3.2.10.

3.2.14 Proximate Composition of Cookie Samples

Moisture, crude protein, fat, ash, and crude fibre of cookie samples were determined using the standard methods described in the AOAC (2012) as explained in section 3.2.6. The carbohydrate content was determined as the weight difference using moisture, crude protein, lipid, crude fibre, and ash content data while the energy value (kcal/100 g) was evaluated by using the Atwater conversion factor (9 × % lipids + 4 × % proteins + 4 × % carbohydrates) (Kobue-lekalake *et al*., 2022).

3.2.15 Mineral analysis of the Cookie samples

Mineral analysis of the cookie samples was determined using the standard methods described in the AOAC (2012) as explained in section 3.2.7.

3.2.16 Determination of beta carotene

Pro-vitamin A content in the cookies was determined using Atomic Absorption Spectrophotometry as explained in section 3.2.8 and described by Honi, (2016) and Fortunatus *et al*., (2021).

3.2.17 Determination of antinutritional factors in Cookie samples

Anti-nutritional factors (hydrogen cyanide, tannin and phytate) in cookie samples were determined as explained in section 3.2.9 and outlined by Ajibola and Olapade, (2021).

3.2.18 Sensory Evaluation of cookie samples

The methods described by Arise *et al*., (2021) and Iwe (2014), were used for evaluating the sensory attributes of the Provitamin A-Bambara cookies. The appearance, taste, aroma, crispness/texture and overall acceptability of the prepared cookie samples (6 samples) were assessed by a 20-member panel of semi-trained judges (average age of 25 comprising of 10 female and 10 male) randomly selected from students of Department of Food Technology, University of Ibadan, who are regular consumers of cookie and are familiar with cookie quality attributes. Sensory attributes of the cookies were scored on a 9-point Hedonic scale. In this scale, 9 represents like extremely; 8-like very much; 7-like moderately; 6-like slightly; 5-niether like nor dislike; 4-dislike slightly; 3-dislike moderately; 2-dislike very much; and 1-dislike extremely.

The evaluation started 2hours after production. Samples were placed on white plates and identified with random three-digit numbers to prevent bias (Ac=305, Bc=311, Cc=817, Dc=502, Ec=104, Fc=174). The panelists were instructed to sip water before and after assessing each product in order to prevent a carry-over effect.

The use of only adults to taste the product intended for both children and adults is due to the fact that difference testing and descriptive analysis are best left to adults who have similar perceptions to those of children, and yet greater cognitive abilities, as required to carry out difference testing, scaling and descriptive analysis (Honi, 2016).

3.2.19 Evaluation of the In-vitro Protein Digestibility of Cookie Samples

The in vitro protein digestibility of cookies was evaluated using three-enzyme pH-drop technique described by Falade and Akeem, (2020). A 6.20 mg of each sample was dissolved in 4 ml distilled water and the pH adjusted to 8.0 with 0.1 N NaOH while stirring at 37 °C in a water bath. A multi-enzyme solution consisting 1.60 mg trypsin, 12.40 mg chymotrypsin and 5.20 mg peptidase per 4 ml of distilled water, was maintained in an ice bath and adjusted to pH 8.0 as earlier described above. A 400 µl aliquot of the multi-enzyme solution was added to the sample solution with

constant stirring at 37 °C. The pH of the solution was recorded 10 min after adding the enzyme solution. The in vitro protein digestibility was then calculated according to equation below;

$$In\ vitro\ protein\ digestibility\ (\%) = 210.464 - 18.103X$$

X = pH of the sample suspension after 10 min digestion with multi-enzyme solution.

3.2.20 Statistical Analysis

All experimental data were expressed as mean ± SD (standard deviation) of duplicate determinations. The data obtained were subjected to one-way analysis of variance (ANOVA) while Duncan's multiple range test (DMRT) was conducted to separate the means at $P<0.05$ (95 % confidence interval). These were achieved using the Statistical Package for Service Solution (SPSS) version 25.0. Data were presented in tabular and graphical forms.

CHAPTER FOUR

4.0 RESULT AND DISCUSSION

4.1 Functional Properties of Flour Blends

Functional properties are the physical and chemical characteristics of a product influencing its behaviour in food system during processing, storage, cooking and consumption (Folaju *et al*., 2018). The results of the functional properties of flour blends from provitamin A cassava (PVAC) and Bambara groundnut (BG) are shown in Table 4.1.

Water absorption capacity (WAC) represents the ability of a product to associate with water under conditions where water is limited. This will not only aid in bulking application (Ubbor *et al.*, 2022) but will also be very useful in bakery products, as this could prevent staling by reducing moisture loss (Okpala and Okoli, 2011). Water absorption capacity ranged from 1.31±0.01 to 1.93±0.16g/g. The water absorption capacity was highest in sample A while sample F had the least. There was no significant ($p>0.05$) difference in the water absorption capacity among most of the flour blends. Although some samples showed significant ($p<0.05$) difference. WAC was found to decrease with increased Bambara groundnut substitution and increased with increased cassava substitution. According to Butt and Batool (2010), protein has both hydrophilic and hydrophobic properties, and can interact with water in foods. Carbohydrates have also been reported to influence water absorption capacity of foods (Adejuyitan *et al*., 2009). It appeared that the starch granules in cassava absorb water more than in the Bambara flour when subjected to similar conditions. This could be due to the fact that about 80-90 percent of the carbohydrate in cassava is starch while Bambara groundnut flour contained 58.88 starch (Ayetigbo *et al*., 2018).

Oil absorption capacity ranged from 0.77±0.06 to 1.40±0.14g/g with sample A having the highest value and sample F the lowest. There was no significant ($p>0.05$) difference in the oil absorption capacity among most of the flour blends. Oil absorption capacity (OAC) has been attributed to the physical entrapment of oil. This is important since fat acts as flavour retainer and increases the mouthfeel of food. It is an indication of the rate at which the protein binds to fat in food formulations (Hasmadi *et al*., 2020). It was observed the oil absorption capacity of the flour blends

Table 4.1: Functional properties of flour blends from pro-vitamin A cassava and Bambara groundnut

Sample Code (PVAC:BG) (%)	Water Absorption Capacity (g/g)	Oil Absorption Capacity (g/g)	Bulk Density (g/ml)	Dispersibility (%)	Swelling Power (%)	Solubility Index (%)	Foaming Capacity (%)	Emulsion Capacity (%)	Least Gelation Concentration (%)
A (100:0)	1.93±0.16^{e}	1.40±0.14^{c}	0.57±0.03^{a}	83.10±0.14^{a}	50.20±0.30^{f}	15.04±0.04^{a}	2.55±0.16^{a}	40.99±0.36^{a}	4.02±0.01^{a}
B (80:20)	1.85±0.00^{de}	1.08±0.11^{b}	0.62±0.02^{b}	84.00±0.42^{b}	46.73±0.16^{e}	16.19±0.23^{b}	9.13±0.42^{b}	46.75±0.03^{b}	3.96±0.07^{a}
C (60:40)	1.76±0.04^{cd}	0.96±0.04^{ab}	0.66±0.02^{c}	84.86±0.23^{c}	41.75±0.04^{d}	16.92±0.02^{b}	12.32±0.35^{c}	50.74±0.80^{c}	6.01±0.04^{b}
D (40:60)	1.64±0.01^{bc}	0.94 ±0.01^{ab}	0.67±0.02^{c}	86.32±0.13^{d}	39.14±0.23^{c}	18.22±0.31^{c}	13.38±0.33^{d}	57.45±0.16^{d}	6.67±0.32^{c}
E (80:20)	1.49±0.01^{b}	0.82 ±0.05^{a}	0.70±0.02^{c}	86.99±0.06^{e}	34.14±0.09^{b}	18.91±0.18^{cd}	17.76±0.57^{e}	60.61±0.58^{e}	7.70±0.10^{d}
F (0:100)	1.31±0.01^{a}	0.77 ±0.06^{a}	0.75±0.03^{d}	87.88±0.11^{f}	31.37±0.05^{a}	19.58±0.61^{d}	21.51±0.25^{f}	72.64±0.28^{f}	8.03±0.01^{d}

Values are Means ± standard deviation of duplicate determinations $^{a-f}$ Means with the same superscripts within the same columns are not significantly ($p>0.05$) different. Where PVAC: Pro-vitamin A Cassava flour, BF: Bambara groundnut flour

increased with increase in yellow root cassava flour. This could be due to increased lipid-lipid interactions. The presence of high-fat content in flours might have affected the oil absorption capacity (OAC) of the composite flours adversely (Chandra *et al*., 2014). Bambara has a relatively higher fat content than cassava. The increased fat content of the flour blends with increase in Bambara might have resulted to its inability to absorb or retain more oil in its structure (Folaju *et al*., 2018). This could however explain the reason why sample F has the lowest oil absorption capacity. High oil absorption capacity will improve mouth feel and flavour retention (Peter *et al*., 2017) and the composite flour with improved oil absorption capacity probably could be used as functional ingredients in foods such as whipped toppings, sausages, chiffon dessert, angel and sponge cakes etc (Ubbor *et al*., 2022). Oil absorption capacity of flour contribute to ensuring stable and uniform pastes and emulsions are formed in production of local confections and baked goods, and as anti-sticking material during cooking of pasta or frying of fishes (Ayetigbo *et al*., 2018). Although, the high OAC of flours might be challenging with respect to shelf life particularly in bakery products (Adebowale and Lawal, 2004).

Bulk density is a measure of the heaviness of flour produced**.** The bulk density ranged from 0.57±0.03 to 0.75±0.03g/ml. Sample A had the least value while sample F had the highest value. There was no significant ($p>0.05$) difference between samples C, D and E. It was observed that bulk density increased with increase in Bambara groundnut. Similar result was recorded by Arise *et al.,* (2018) of an increase in bulk density with increased substitution of Bambara flour in maize. Higher bulk density is desirable since it offers greater packaging advantage as greater quantity of flour can be packed within a constant volume (Agbemafle, 2019). High bulk density increases the rate of dispersion, which means better reconstitution (Aniedu and Omodamiro, 2012). The decrease observed in bulk density of the flour blends with increased addition of yellow root cassava flour could be as a result of the reduced particle size of the flour blends (Folaju *et al*., 2018). This is similar to the observation of Danbaba *et al.* (2014) where increasing level of cassava flour substitution in 100% rice flour decreases its bulk density. Low bulk density is however a desirable attribute for preparation of complementary foods (Folaju *et al*., 2018) because loose bulk density facilitates sufficient energy-nutrient intake per feed of a child and easy digestibility (Zakari *et al*., 2018; Oluwole *et al*., 2022) and is appropriate in bakery industry (Aniedu and Omodamiro, 2012). In addition, low bulk density of flour samples is a good attribute when determining transportation and storability and since the products could be easily transported and distributed to required

location (Ubbor *et al*., 2022). Important to note is that the value (0.75g/ml±0.03) for bulk density of sample F which is 100 percent Bambara flour falls within the range (0.543 g/mL to 0.816 g/mL), reported by Du *et al*. (2014) as the bulk density for legume flours.

The dispersibility percentage is an indicator of good water absorption capacity of flours and an indicator of good quality of the gel. The dispersibility of flour which gives an indication of the suspension of the particles in water has been reported to be associated with fine particle size (Kacou *et al*., 2018). The dispersibility of the flour blends ranged from 83.10±0.14 to 87.88±0.11%. Sample A had the least value while sample F had the highest value. Although there was significant ($p<0.05$) difference among the flour blends in terms of dispersibility, the both flours used in this study were found to have high value of dispersibility, with an increase with increased levels of Bambara groundnut substitution. According to Sara *et al.*, (2008) a high value of dispersibility increases the emulsifying and foaming properties of proteins. Flours that have good dispersibility values give a reconstitution capacity of a fine and consistent dough (Omodamiro *et al*., 2012). The values (87.8%) for sample A (100% yellow root cassava flour) reported in this study is close to the range (80–86%) reported by Awoyale *et al*., (2015).

The swelling powers of the flour blends ranged between 31.37±0.05 to 50.20±0.30%. Sample A had the highest swelling power while sample F had the lowest swelling power. There was significant difference ($p<0.05$) in the swelling powers of the flour blends. Swelling power is the spontaneous uptake of water by protein or starch matrix and the ability to hold the water in its structures before and during gelatinization (Folaju *et al*., 2018). It further indicates the water binding capacity of the starch or mixture in a product; correlates with final product quality and as well provides an indication of the viscous load likely to be encountered by a mixing cooker (Ingbian and Adegoke, 2007). It was observed that an increase in swelling capacity occurred due to an increase in the yellow root cassava flour substitution. This is expected because cassava has been reported to have a high starch content of about 82.39% to 84.04% (Agbemafle, 2019). The increase in swelling power could be ascribed to high level interaction between water molecules and amylopectin fraction of the root starch granules resulting to high gel strength and elasticity of the flour blends (Folaju *et al*., 2018). Moreover, the swelling power of flour samples are often related to their protein and starch contents. Hence, higher protein content in flour may cause the starch granules to be embedded within a stiff protein matrix which subsequently limits the access

of the starch to water and restricts the swelling. Flours lower in protein and higher in total starch content have higher swelling ability (Agbemafle, 2019). Furthermore, Abera and Rakshit (2003) reported that the swelling ability of starch is inhibited by its lipid content. This could also be partly the reason for the higher swelling power observed in yellow root cassava than Bambara groundnut since the former is associated with higher starch content with low fat content while the latter is associated with higher protein and fat content as stated in the literature and verified by this study.

The solubility index of the flour blends ranged from 15.04±0.04 to 19.58±0.61% with sample F having the highest value and sample A having the least value. There was no significant ($p>0.05$) difference between samples B and C, samples D and E and samples E and F. But other samples showed significant ($p<0.05$) differences. This study however recorded relatively high solubility index for the flour blends. Solubility of flour is an indicator of quality, therefore the high values observed in the flour blends suggest that they could be very digestible and therefore could possibly be suitable for infant food formulations (Agbemafle, 2019). Denaturation of proteins has been shown to increase their solubility (Kinsella, 1976; Agbemafle, 2019).

The foaming capacity of a protein refers to the amount of interfacial area that can be created by the protein. The foaming capacity of the flour blends ranged between 2.55±0.16% (Sample A) and 21.51 ± 0.25% (F). There was significant difference ($p<0.05$) between the foaming capacity of all the flour blends. An increase was observed with increased substitution of Bambara groundnut. According to Agbemafle, (2019), high protein content of a flour increases the foaming capacity of the flour and cassava do not contain considerable high amounts of protein, a good foaming agent. This accounted for the relatively low values recorded for sample A (100:0). The increase in foaming capacity might be due to a decrease in surface tension of the air and water interface, leading to the absorption of soluble protein molecules, thereby permitting hydrophobic interactions (Chinma *et al*., 2009). This is plausible since the foaming capacity of a food material depends on the surface-active properties of its protein (Agbemafle, 2019; Hasmadi *et al*., 2020). Foams are used to improve texture, consistency and appearance of foods (Akubor, 2007; Kacou *et al*., 2018). The capacity of proteins to form stable foams with gas by forming impervious protein films is an important property in cakes, soufflés, whipped toppings, fudges, ice creams and marshmallows (Agbemafle, 2019).

Emulsion capacity indicates the amount of oil that can be emulsified by protein dispersion. Therefore, it depends on oil content and protein concentration of a product (Folaju *et al*., 2018). The emulsion capacity of flour blends ranged between 40.99±0.36% (sample A) and 72.64±0.28 % (sample F). There was significant difference ($p<0.05$) between all the samples. Protein being the surface active agents can form and stabilize the emulsion by creating electrostatic repulsion on oil droplet surface (Kacou *et al*., 2018). This could explain the reason for the high emulsion capacity observed with increased Bambara groundnut substitution as it records a remarkably high protein content based on the study. The increase observed indicate high interaction between protein and fat content of the flour blends (Folaju *et al*., 2018). High emulsion capacity is an indication of the flours being useful as an additive for the stabilization of fat emulsion in the production of sausage, soup and cake (Eltayeb *et al*., 2011).

The least gelation concentration (LGC), which is defined as the lowest protein concentration at which gel remained in the inverted tube, was used as an index of gelation capacity. Least gelation concentration is used to measure the ability of the protein to form a gel, whereby a lower least gelation concentration suggests a better gelling capacity (Abu *et al*., 2005). The LGC of the flour blends ranged between 4.02± 0.01% (sample A) and 8.03 ± 0.01 % (sample F). There was no significant ($p>0.05$) difference between samples A and B and samples E and F. However, all other samples showed significant ($p<0.05$) difference. The presence of carbohydrates such as lactose, maltose, and sucrose are reported to decrease the thermodynamic affinity of the protein for an aqueous solution and magnifies the magnitude of the interaction between protein molecules, thus improving the gelling capacity (Adebowale and Adebowale, 2008). This accounted for the relatively low and hence better gelling capacity observed with sample A. The result obtained for LGC is in agreement with the observation of Kacou *et al.,* (2018) that the flour which was higher in starch content took lowest temperature for gelatinization. According to Maninder *et al*., (2007), LGC for various legume flours ranged from 12% to 14%. The result (8.03%) of this study for sample F which is 100% Bambara flour (a legume) was not in agreement with this range. Increase in Bambara flour substitution resulted in a slight increase in LGC, however, the flour blends all recorded relatively low values for LGC. The primary function of gel in foods is to bind or solidify the free water (Onwuzuruike *et al*., 2020). These may find useful applications in food systems such as sausage emulsions, custard type puddings, and sauces, which require thickening and gelling (Hasmadi *et al*., 2020).

4.2 Proximate Composition of Flour Blends

The proximate composition and energy contents of composite flours from the blends of Provitamin A Cassava and Bambara groundnut flour are given in Table 4.2. Moisture content of food gives an indication of the shelf-life and nutritive values (Agbemafle, 2019). The moisture contents ranged from 3.94±0.02% to 7.10±0.07% where sample F had the lowest value and A had the highest value respectively. There were significant differences ($p<0.05$) in moisture content of some flour blends whereas for some samples like E and F; C and D, no significant ($p>0.05$) difference was observed. The moisture contents of the flour blends decreased with increasing level of Bambara groundnut flour. Similar observations were reported by Orhevba and Mbamalu, (2017) for moisture content of sample F which is 100% Bambara (3.80-4.42%) while that of 100% cassava flour (sample A) is slightly higher than the value (5.95%) reported by Agbemafle, (2019). Different drying kinetics of flours may be the cause of the variances (Folaju *et al*., 2018). This may also be attributed to the differences in varieties of cassava used for the work. The moisture contents of the flour blends were found to be below 10% maximum recommended by Standard Organization of Nigeria (SON) for the long storage of flour (Sanni *et al*., 2005; Adebowale *et al*., 2011), and with appropriate packaging, this can favor long shelf-life storage (Kobue-lekalake *et al*., 2022)

Crude protein content ranged from 2.00±0.02% to 20.94±0.09% sample A having the lowest value and sample F the highest value respectively. These results confirmed by statistical analysis, for which significant differences ($p < 0.05$) were observed between the protein contents in the flour blends. An increase in protein content was observed with increase in Bambara groundnut flour. It is possible that the increase in protein content is due to the high protein content of Bambara groundnut which was reported to be within the range of 18% – 23% (Arise and Malomo, 2021; Damfami and Oat, 2020; Tan *et al*., 2020). This indicates that supplementing Provitamin A cassava flour with Bambara bean flour would greatly improve the protein nutritional quality of food products produced from the composite flour. For people with protein-deficit malnutrition, particularly children, the flour blends would be a suitable supply of protein.

Crude fat contents of the flour blends ranged from 0.83±0.03% to 6.56±0.16% with sample F having the highest value while sample A having the lowest value. There were significant differences ($p<0.05$) among samples. The increase in fat content may be due to incorporation of

4.2 Proximate Composition of Flour Blends of pro-vitamin A cassava and Bambara groundnut

Sample Code (PVAC:BG) (%)	Moisture (%)	Protein (%)	Crude Fat (%)	Ash (%)	Crude Fiber (%)	Carbohydrate (%)	Energy value (Kcal/100g)
A (100:0)	7.10±0.07^{e}	2.00±0.02^{a}	0.83±0.03^{a}	1.32±0.01^{a}	4.67±0.05^{f}	84.14±0.03^{f}	352.01±0.28^{a}
B (80:20)	5.69±0.35^{d}	7.46±0.32^{b}	1.08±0.01^{b}	1.95±0.04^{b}	4.31±0.06^{e}	79.53±0.04^{e}	357.66±1.57^{b}
C (60:40)	4.76±0.08^{c}	9.78±0.33^{c}	2.42±0.05^{c}	2.17±0.22^{b}	4.20±0.01^{d}	76.69±0.69^{d}	367.60±1.00^{c}
D (40:60)	4.41±0.05^{bc}	13.34±0.41^{d}	3.72±0.04^{d}	3.02±0.01^{c}	3.64±0.03^{c}	71.88±0.44^{c}	374.32±0.43^{d}
E (80:20)	4.19±0.05^{ab}	16.88±0.18^{e}	4.03±0.01^{e}	3.86±0.17^{d}	2.91±0.01^{b}	68.15±0.40^{b}	376.33±0.94^{d}
F (0:100)	3.94±0.02^{a}	20.94±0.09^{f}	6.56±0.16^{f}	4.30±0.11^{e}	1.32±0.02^{a}	62.96±0.33^{a}	394.58±0.53^{e}

Values are Means ± standard deviation of duplicate determinations $^{a-f}$ Means with the same superscripts within the same columns are not significantly ($p>0.05$) different. Where PVAC: Pro-vitamin A Cassava flour, BF: Bambara groundnut

Bambara. Bambara has been documented to have a relatively higher fat content of about 6.60% (Arise and Malomo, 2021) and a range of 6.5 to 8.5% as reported by Jideani and Jideani, (2021). As a result, the energy density of the flour blends would rise, and prolonged consumption of the product form may result in stomach retention. The fat content of the blended flour samples was generally low, and such product may contribute to the prevention of obesity, diabetes mellitus type II and various cardiovascular disease development in humans (Ferreira, *et al*., 2021). Moreover, this will enhance the storage life of the flour due to the lowered chance of rancid flavour development (Agbemafle, 2019).

Ash content ranged from 1.32±0.01% to 4.30±0.11% where sample A had the lowest value and sample F had the highest value respectively. No significant difference ($p>0.05$) exists between B and C in terms of the ash content but significant differences ($p<0.05$) was observed for the other samples. The ash content of a given food provides some insight to its mineral content and could also be employed as an index of fibrous contamination in flour products (Adewuyi and Abu, 2014). BG flour is known to bear a higher ash content (3.6–3.84%) (Kaptso *et al*., 2015). The increase in ash content with increase in Bambara flour might be indicative of increases in mineral content.

The amount of cellulose, hemicelluloses, and lignin in a food is determined by its crude fibre content (Agbemafle, 2019). Crude fibre content ranged from 1.32±0.02% to 4.67±0.05% where sample F had the lowest value and sample A had the highest value respectively. There were significant differences ($p<0.05$) among the samples. The fiber content in the blended flour samples is less than 5%, and the food processed from such flour can meet the Codex Alimentarius Commission (2013) recommendation of less than 5% fiber in the complementary weaning foods.

Carbohydrate content of the flour blends ranged from 62.96±0.33% to 84.14±0.03% with sample A having the highest value while sample F the lowest value respectively. The values showed significant differences ($p<0.05$) among the flour blends. The results showed that sample A (100% cassava flour) has a higher carbohydrate content than flour sample produced using Bambara as expected based on the literature. High carbohydrate contents indicate that a food is an excellent source of energy. The high carbohydrate values obtained in this study correlates with the assertion that cassava could be utilized as a reliable food and energy security crop as proposed by FAO (2008). On the other hand, the decreased utilizable carbohydrate content in the blended flour as the BG increased could be due to the fact that BG is low in its carbohydrate content (57.9 to 61.7%)

(Kaptso *et al*., 2015) as compared to cassava flour (80–90% dry basis) (Ayetigbo *et al*., 2018). A flour product with decreased utilizable carbohydrates and increased fiber contents is beneficial because a low glycemic diet is known to suppress diabetes mellitus type II disease. In this respect, BG was reported to suppress hyperglycemia and hyperlipidemia (Megwas *et al*., 2021), which are risk factors for the development of obesity, diabetes and cardiovascular diseases (Bays, 2020). Indeed, because of high soluble and insoluble dietary fibers and the associated phenolic compounds in the Bambara groundnut, its use in functional foods and as nutraceuticals are purported to suppress diabetes mellitus type II, cardiovascular diseases, and cancers (Okafor *et al*., 2022; Kobue-lekalake *et al*., 2022).

The energy content in the blended flours ranged from 352.01±0.28 to 394.58±0.53kcal/100 g. There was significant ($p<0.05$) difference in the energy contents of the flour blends.

4.3 Mineral Composition and Beta Carotene Content of Flour Blends

Table 4.3 presents the result of mineral composition and beta-carotene content of Provitamin A Cassava and Bambara groundnut flour blends. Calcium contents of flour mixtures ranged from 184.43±0.01 to 253.54±0.03mg/100g with sample A having the highest, while F having the least. The calcium contents of blend increased as quantity of PVAC increased. Sodium content ranged from 17.43±0.03 to 20.73±0.02mg/100g with sample A having the highest value while sample F has the least value. Magnesium contents of flour blends varied from 35.35±0.03 to 68.32±0.01 mg/100g with sample F having the highest, while A having the least. Phosphorus and Potassium contents of the flour blends varied from 338.05±0.02 to 554.03±0.02mg/100g and 158.69±0.06 to 193.22±0.01mg/100g with sample F having the highest, while A having the least respectively. Significant ($p<0.05$) differences existed between them except for samples E and F which showed no significant ($p>0.05$) difference in their sodium content.

The iron and zinc contents of the blends ranged from 0.86±0.02 to 8.59±0.05mg/100g and 0.76±0.01 to 5.03±0.01mg/100g, respectively. The flour blends were significantly ($p<0.05$) different in their iron and zinc compositions respectively. With exceptions for samples C and D which showed no significant ($p>0.05$) difference in their zinc contents. The value of iron for sample F (100% Bambara flour) is slightly higher than the value (5.45 mg/100 g) reported by Abdualrahman *et al*., (2019) but in the same range with the 8.8 mg/100 g reported by Semba *et al*., (2021).

Table 4.3 Mineral Composition and β-carotene content of Flour Blends of pro-vitamin A cassava and Bambara groundnut

Sample Code (PVAC:BG) (%)	Calcium (mg/100g)	Sodium (mg/100g)	Magnesium (mg/100g)	Phosphorus (mg/100g)	Potassium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	B-carotene (μg/g)
A (100:0)	253.54±0.03^{f}	20.73±0.02^{e}	35.35±0.03^{a}	338.05±0.02^{a}	158.69±0.06^{a}	0.86±0.02^{a}	0.76±0.01^{a}	7.72±0.18^{e}
B (80:20)	247.53±0.70^{e}	20.23±0.31^{d}	39.28±0.06^{b}	381.93±0.70^{b}	163.23±0.01^{b}	2.18±0.03^{b}	1.16±0.00^{b}	7.19±0.21^{d}
C (60:40)	233.21±0.65^{d}	19.67±0.18^{c}	43.74±0.04^{c}	410.98±0.01^{c}	167.33±0.04^{c}	3.98±0.01^{c}	2.21±0.01^{c}	6.89±0.07^{cd}
D (40:60)	219.19±0.01^{c}	18.56±0.01^{b}	49.16±1.24^{d}	446.34±0.67^{d}	180.00±0.01^{d}	5.49±0.00^{d}	2.25±0.01^{c}	6.81±0.04^{c}
E (80:20)	198.51±0.03^{b}	17.15±0.08^{a}	56.61±0.19^{e}	492.26±0.03^{e}	184.80±0.86^{e}	7.64±0.06^{e}	3.94±0.06^{d}	5.22±0.16^{b}
F (0:100)	184.43±0.01^{a}	17.43±0.03^{a}	68.32±0.01^{f}	554.03±0.02^{f}	193.22±0.01^{f}	8.59±0.05^{f}	5.03±0.01^{e}	4.04±0.03^{a}

Values are Means ± standard deviation of duplicate determinations $^{a-f}$ Means with the same superscripts within the same columns are not significantly ($p>0.05$) different. Where PVAC: Pro-vitamin A Cassava flour, BF: Bambara groundnut flour

The range of zinc content in BG (2.14 to 19.73 mg/100 g) as reported by Halimi *et al*., (2019) agrees with the value (8.59mg/100g) obtained from this study. Blending with BG at 20% showed an increase of 153.5% iron content from the 100% cassava flour sample (2.18-0.86/0.86 x 100/1); this is expected because iron content was found to be high in BG as reported in the literature. For zinc, blending with Bambara groundnut at 20% showed an increase of 52.6% zinc content from the 100% provitamin A cassava flour sample (1.16-0.76/0.76 x 100/1) because the zinc content in BG is reported to be high (2.14 to 19.73 mg/100 g) (Halimi *et al*., 2019).

The beta-carotene content in the blended flour samples were ranged from 4.04±0.03 to 7.72±0.18 µg/g (Table 4.3). Among the blended flours, a significant increase ($p < 0.05$) was observed for the highest PVAC blending; hence, sample A has the highest value while sample F has the lowest. This shows the major beta-carotene contribution to the blended flours is from the Provitamin A cassava flour. Hence, food products produced from this flour will be beneficial in supplying pro-vitamin A carotenoids. Vitamin A continues to be an essential part of human nutrition, as it helps with eyesight, cell differentiation, the synthesis of glycoprotein, reproduction, and general growth and development (Okoye and Ezeugwu, 2019)**.**

4.4 Anti-nutritional Factors of Flours (Main ingredients)

Anti-nutritional factors in foods are mainly responsible for negatively impacting the absorption of nutrients and micronutrients in the digestive system which may interfere with the functioning of certain organs (Gemede and Ratta 2014; Unigwe *et al*., 2018). Anti-nutritional factors of flours are depicted in Table 4.4. The hydrogen cyanide (HCN) contents of flours are 3.47±0.02mgHCNeqv/100g for provitamin A cassava flour and 1.30±0.11 mg HCN eqv /100g for Bambara groundnut flour. The value of HCN reported in this study for provitamin A cassava flour is higher than the value (2.45 HCN eqv /100g) reported by Olapade and Adegboye, (2018) but falls within the range (3.17-6.53 HCN eqv /100g) reported by Adebowale *et al*., (2011). The differences could be attributed to the variety of cassava root used for the work. However, The HCN contents of the flours (3.17−6.53 mg HCNeqv/100 g) were within the values recommended by SON (<10 mg HCNeqv / 100g) (Sanni *et al*., 2005; Adebowale *et al*., 2011).

The tannin compositions of the flours are 0.21±0.00mg/100g and 0.33±0.01 mg/100g for provitamin A cassava flour and Bambara groundnut flours respectively. The value reported for tannin of Bambara groundnut flour in this study agrees with the range 0.2-6.2mg reported by

Table 4.4: Anti-nutritional composition of flours (main ingredients) (mg/100g)

Flour sample	Hydrogen Cyanide	Tannin	Phytate
PVAC	3.47±0.02	0.21±0.00	2.52±0.01
BG	1.30±0.11	0.33±0.01	2.12±0.06

PVAC=Provitamin A cassava flour; BG= Bambara groundnut flour

Values are means ± standard deviation of duplicate determination

Unigwe *et al*., (2018) which is relatively low. However, the presence of tannins in the food has a negative effect on protein digestibility in animals and humans by either making protein partially unavailable or inhibiting digestive enzymes (Yao *et al*., 2015). Tannins are known to be present in food products and to inhibit the activities of trypsin, chemotrypsin, amylase, and lipase, decrease the protein quality of foods and interfere with dietary iron absorption. If tannin concentration in the diet becomes too high, microbial enzyme activities including cellulose and intestinal digestion may be depressed (Unigwe *et al*., 2018).

Phytate (also known as inositol hexakisphosphate - IP_6) is one of the most powerful anti-nutritional factors in plant feeds and is present in considerable quantities within major legumes and oilseeds (Akande *et al*., 2010; Gemede and Ratta 2014). Phytic acid is known to inhibit the action of gastrointestinal tyrosinase, trypsin, pepsin, lipase, and amylase (Unigwe *et al*., 2018). The phytate composition of the flours are 2.52±0.01mg/100g for provitamin A cassava flour and 2.12±0.06mg/100g for Bambara groundnut flour. The phytate content recorded for Cassava flour in this study slightly agrees with the range 2.63-3.00mg/100g reported by Adebowale *et al*., (2011). The value for Bambara groundnut flour does not agree with the findings of Madukwe *et al*., ((2013) of 0.001mg/g. This could be as a result of the variety of Bambara groundnut used for the work.

4.5 Colour of Flours (Main ingredients)

The result of the colour variations in flours of Provitamin A Cassava and Bambara groundnut is presented in Table 4.5. The colour of flour has been shown to impact on the acceptability of end product and uniqueness of numerous flour criteria desired by consumers (Ajibola, 2020). Variations in colour of flours are 83.14 and 80.47 for l*, 0.47 and 1.39 for a* and 15.41 and 13.94 for b* values of provitamin A cassava (PVAC) and Bambara groundnut (BG) flours respectively. Provitamin cassava flour had greater lightness (l*) (83.14), than Bambara flour (80.47). BG flour was higher in redness (1.39) than PVAC flour (0.47).

Yellowness (b*) of PVAC flour (15.41) was higher whereas BG flour had lesser yellowness (13.94). This might be due to higher Beta-carotene in PVAC roots as stated in the literature. Apart from Beta-carotene, other carotenoids, such as lutein, contribute to the overall color of colored-flesh cassava and Cassava flour made from cassava root retains the color of the root flesh (Ayetigbo *et al*., 2018).

Table 4.5: Colour Variations of Flours (Main ingredients)

Flour Samples	Lightness (l*)	Redness (a*)	Yellowness (b*)
PVAC	83.14±0.07	0.47±0.04	15.41±0.01
BG	80.47±0.01	1.39±0.02	13.94±0.01

PVAC=Provitamin A cassava flour; BG= Bambara groundnut flour

Values are means ± standard deviation of triplicate determination

4.6: Physical Properties of Cookies

Physical properties of cookies are essential quality indicators (Arise *et al.*, 2021). The physical characteristics of cookies prepared from flour blends of Provitamin A cassava flour and Bambara groundnut flour are presented in Table 4.6.

The weight of the cookies ranged from 8.31 to 9.02 g; cookie sample Fc was the heaviest (9.02g) and bulkiest among the samples, while the cookie sample Ac had the least weight (8.31g). The weight of composite cookies differed significantly ($p<0.05$). Addition of Bambara flour to provitamin A cassava flour caused a significant ($p < 0.05$) increase in the weight of cookies. The increase in weight of composite cookies could be attributed to high bulk density of Bambara flour than cassava as observed in this study. This is plausible since the mass, size and geometry of individual particles are essential components of bulk density (Arise *et al.*, 2021). The higher bulk density of cassava-Bambara flour blends could also account for higher weight of composite cookies. Apotiola and Fashakin, (2013) and Chinma *et al.*, (2012) also attributed increase in weight of composite cookies to the high bulk density of higher protein substitutes; soyflour and defatted sesame flours respectively.

The diameter of cookies ranged from 4.08 to 4.53cm with cookie sample Ac having the highest value while cookie sample Fc has the least value. Addition of Bambara flour to provitamin A cassava flour in the cookie formulation caused a significant ($p<0.05$) decrease in the diameter. The diameter of the cookies is important in determining the packaging material to be used for the cookies (Ubbor *et al.*, 2022).

The thickness followed a similar trend as the weight of composite cookies, which significantly ($p < 0.05$) increased from 0.54 to 0.68 cm for cookie sample Ac and cookie sample Fc respectively. The higher bulk density of Bambara could have affected its thickness positively. Moreover, a higher protein content implies a decrease in spread factor and decrease in spread factor is concurrent to an increase in thickness since the both variables are inversely proportional (spread ratio=diameter/thickness).

A similar decreasing trend as the diameter of the cookies was recorded for the spread ratio of the cookie samples. The spread ratio (diameter / thickness) of cookies ranged from 6.04 to 8.46; cookie sample Ac had the highest spread factor while cookie sample Fc had the least. It was observed that

Table 4.6: Physical Properties of Cookie Samples

Cookie Samples	Weight (g)	Diameter (cm)	Thickness (cm)	Spread ratio	Breaking Strength (kg)
Ac	8.31 ± 0.01[a]	4.53 ± 0.01[f]	0.54 ± 0.01[a]	8.46±0.10[d]	5.66±0.01[f]
Bc	8.46 ± 0.02[b]	4.46 ± 0.01[e]	0.52 ± 0.01[a]	8.66±0.14[d]	5.41±0.01[e]
Cc	8.61 ± 0.01[c]	4.38 ± 0.00[d]	0.58 ± 0.01[b]	7.55±0.18[c]	5.07±0.02[d]
Dc	8.69 ± 0.05[d]	4.29 ± 0.01[c]	0.64 ± 0.01[c]	6.80±0.14[b]	4.85±0.03[c]
Ec	8.92 ± 0.02[e]	4.18 ± 0.01[b]	0.64 ± 0.01[c]	6.59±0.05[b]	4.33±0.01[b]
Fc	9.02 ± 0.01[f]	4.08 ± 0.02[a]	0.68 ± 0.01[d]	6.04±0.09[a]	4.13±0.01[a]

Values are means ± standard deviation of duplicate determination

Mean values within the same column followed by different superscripts are significantly different ($P<0.05$).

the spread factor of composite cookies significantly ($p < 0.05$) decreased with increasing level of substitution with Bambara flour. Apotiola and Fashakin, (2013) and Chinma *et al*., (2012) also reported a decrease in spread factor with increased protein in the cookies. According to Nasir *et al*., (2010), this is plausible because the increased number of hydrophilic sites available due to increased protein content competes for the limited free water in the cookie dough resulting to a decrease in spread factor. Cookies with higher spread ratios are considered most desirable (Ayo *et al*., 2007). This is however contrary to the assertion by Olapade and Adeyemo (2014). Therefore, the control of cookies spread ratio is a serious problem encountered during production; cookies that spread so much cannot be filled into the package and those that spread slightly causes slack fill or excess height for package, thus creating havoc on the packaging line (Ikuomola *et al*., 2017).

The breaking strength of the cookies ranged between 4.13 and 5.66kg; cookie sample Ac had the highest value (5.66kg), while cookie sample Fc had the least value (4.13kg). The breaking strength is referred to the force required to break the cookies (Ikuomola *et al*., 2017). The breaking strength significantly ($p < 0.05$) decreased as the substitution level of Bambara flour increased in the formulation. The reduction could be attributed to the carbohydrate/starch content of Bambara flour which may not be as hard/strong like that of cassava flour. Hardness of cookies is caused by the interaction of proteins and starch by hydrogen bonding (Ikuomola *et al*., 2017). Comparatively, the finding agrees with the reports of Ayo *et al*., (2007) for the utilization of composite flours in cookies to reduce its break strength. Also, Adebowale *et al*., (2012) reported that increase in rigidity is due to increase in carbohydrate/starch granules, which is responsible for gel and structure formation in baked goods. Higher breaking strength obtained in cookies made from composite flours indicates greater hardness of cookies structure (Banusha and Vasantharuba, 2014; Offia-Olua and Akubuo, 2019).

The results obtained for physical properties of PVAC-BG cookies are in close agreement with those of Offia-Olua and Akubuo, (2019) and but lower than the values reported by Chinma *et al*., (2012) and Arise *et al*., (2021).

4.7: Colour Variations of Cookie Samples

Colour is an important organoleptic quality attribute, which is considered in the acceptance of food products (Ayetigbo *et al*., 2018). The colour parameters (l*, a*, and b*) of the cookies' outer surface (crust) are shown in Table 4.7.

Table 4.7: Colour Variations of Cookie Samples

Cookie Samples	Lightness (l*)	Redness (a*)	Yellowness (b*)
Ac	65.64 ± 0.49^{d}	0.96 ± 0.10^{a}	17.94 ± 0.04^{e}
Bc	64.01 ± 0.50^{c}	1.47 ± 0.09^{b}	17.28 ± 0.11^{d}
Cc	63.82 ± 0.28^{c}	1.42 ± 0.04^{b}	17.41 ± 0.05^{d}
Dc	60.55 ± 0.84^{b}	2.29 ± 0.05^{c}	17.04 ± 0.03^{c}
Ec	57.73 ± 0.55^{a}	3.18 ± 0.11^{d}	16.87 ± 0.15^{b}
Fc	57.44 ± 0.72^{a}	3.41 ± 0.32^{d}	16.39 ± 0.03^{a}

Values are means ± standard deviation of triplicate determination

Mean values within the same column followed by different superscripts are significantly different ($P<0.05$).

The Commission Internationale de l'Eclairage (CIE) L*, a*, b* colour system was employed to measure the surface color of cookie samples. The L*, a* and b* values are measure of extent of lightness, redness-greenness and yellowness-blueness of the surface color of a material (Eke *et al*., 2009). L* values ranged from 57.44 to 65.64 with cookie sample Ac having the highest value and therefore the lightest while cookie sample Fc had the lowest value. The a* values ranged from 0.96 to 3.41 with sample Fc having the highest value and sample Ac the lowest. The b* values ranged from 16.39 to 17.94 with sample Fc having the lowest value while sample Ac had the highest value.

The surface of the cookie sample Ac was significantly ($p<0.05$) lighter (L= 65.64) and yellower (b=17.94) than in cookie sample Fc made with highest substitution of BG flour which had its values of lightness and yellowness as L=57.47 and b=16.39 respectively. The result showed that the addition of BG flour decreased the L* and b* values and increased the a* value of the cookies. The addition of the BG flour significantly ($p<0.05$) changed the color from yellow to red. Further substitution communicated darkness to the cookies' surface. This can be due to Maillard reactions that occurred in baking via reactions between reducing sugar and amino acids (Nwadi *et al*., 2020). It is however, expected that the cookie samples with increased substitution of BG flour will respond to Maillard reaction more since they have higher protein quality than cassava flour as stated in the literature.

4.8: Proximate Composition of Cookies

Table 4.8 shows the proximate composition of cookies produced from blends of PVAC and BG. The proximate composition result showed that protein, fat and ash increased as the proportion of Bambara flour increased ranging from 3.46±0.02 to 16.33±0.03%, 21.66 ±0.02 to 24.63±0.02% and 2.64±0.02 to 4.26±0.01% respectively. This indicates that supplementing PVAC flour with Bambara groundnut flour would greatly improve the protein quality of cookies since that was the focus of the study. This could obviously be due to the significant quantity of protein in the Bambara groundnut flour (20.94%) as observed in this study. These findings were in agreement with the work of Honi (2016) on the development of orange fleshed sweet potato and Bambara groundnut-based snacks for school children. This similar observation was made in a research study by Oluwole and Karim, (2005), which showed an increase in the protein content with corresponding increase in the proportion of Bambara flour supplementation in biscuit production from cassava-

Table 4.8: Proximate Composition of Cookies from Flour Blends of pro-vitamin A cassava and Bambara groundnut

Sample Code (PVAC: BG) (%)	Moisture (%)	Protein (%)	Crude Fat (%)	Ash (%)	Crude Fiber (%)	Carbohydrate (%)	Energy value (Kcal/100g)
Ac (100:0)	6.74±0.04^{f}	3.46±0.02^{a}	21.66±0.02^{a}	2.64±0.02^{a}	2.98±0.04^{f}	62.54±0.03^{f}	458.88±0.01^{a}
Bc (80:20)	6.43±0.03^{e}	4.13±0.01^{b}	22.35±0.02^{b}	2.97±0.03^{b}	2.67±0.00^{e}	61.46±0.05^{e}	463.45±0.33^{b}
Cc (60:40)	6.04±0.03^{d}	6.85±0.04^{c}	22.74±0.02^{c}	3.22±0.02^{c}	1.99±0.02^{d}	59.18±0.04^{d}	468.72±0.22^{c}
Dc (40:60)	5.95±0.02^{c}	9.04±0.02^{d}	23.86±0.05^{d}	3.54±0.05^{d}	1.32±0.02^{c}	56.32±0.12^{c}	476.10±0.05^{d}
Ec (80:20)	5.83±0.02^{b}	12.51±0.03^{e}	24.02±0.01^{e}	4.02±0.01^{e}	0.67±0.00^{b}	52.96±0.02^{b}	478.04±0.07^{e}
Fc (0:100)	5.75±0.04^{a}	16.33±0.03^{f}	24.63±0.02^{f}	4.26±0.01^{f}	0.61±0.01^{a}	48.44±0.01^{a}	480.69±0.28^{f}

Values are Means ± standard deviation of duplicate determinations $^{a-f}$ Means with the same superscripts within the same columns are not significantly ($p>0.05$) different. Where PVAC: Pro-vitamin A Cassava flour, BF: Bambara groundnut flour

wheat-bambara flour blends. Cookie sample Ac produced from 100% Provitamin A cassava flour was found to be the least in protein content (3.46%), which is expected because cassava is a poor source of protein (Oluwole and Karim, 2005; Ayetigbo *et al*., 2018). As Bambara groundnut substitution increased, an increase in protein content was observed, indicating a fortifying effect of Bambara groundnut flour on the cookies. Fortification of the cookies with up to 40% Bambara flour substitution resulted in about 98% increase (6.85-3.46/3.46 x 100/1=97.97%) in the protein content of the formulated cookie. It was however observed that the protein content of cookies was lower than that recorded from the respective flour blends. The decrease in the protein content of the flour after cookie production may be attributed to heat and Maillard browning (Nwadi *et al*., 2020).

Although fat acts as flavour retainer and helps to improve sensory qualities of baked products (Ikepeme *et al*., 2008); increases the energy content of foods (Abayomi *et al*., 2013); as well as improves bioavailability of beta carotene content of the food therein (Hailu, 2016). However, high levels of fat in food products should be ≤25% (Ihekoronye and Ngoddy, 1985), since this could lead to rancidity in foods and development of unpleasant and odorous compounds and could also mean reduced storage potential (Ihekoronye and Ngoddy, 1985; Ikuomola *et al*., 2017; Agbemafle, 2019). It was observed from this study that all the cookie samples had fat content (21.66 to 24.63%) below this recommended level of fat in food products.

The ash content of a food sample gives an expression of the mineral composition of the sample (Agbemafle, 2019). The increase in ash content with resultant increase in Bambara groundnut could mean higher mineral content. There were significant differences ($p<0.05$) in the protein, fat and ash content of the cookie samples respectively.

However, an opposite trend was observed for moisture, crude fibre and carbohydrate which decreased with increased Bambara flour substitution ranging from 5.75±0.04 to 6.74±0.04%, 0.61±0.01 to 2.98±0.04% and 48.44±0.01 to 62.54±0.03% respectively. The decrease observed could be the consequent result of increasing substitution level of Bambara groundnut flour in the cookies. Moisture content of food gives an indication of the shelf-life and nutritive values (Agbemafle, 2019). All the cookie samples had good moisture contents and hence have the potential for better shelf life. There were significant differences ($p<0.05$) in the moisture content,

crude fibre and carbohydrate content of the cookie samples respectively. With exception for cookie samples Ec and Fc which showed no significant difference ($p>0.05$) in their crude fibre content.

Energy content is a parameter used to determine the quality of food especially for formulations designed for adult with high energy requirements. Food energy is the amount of calorie available from food that is available through oxidation. Fats have the greatest amount of food energy 9kcal/g while proteins and carbohydrates have about 4kcal/g (Abayomi *et al*., 2013). The calorie content of the cookies has been increased from 458.88±0.01 to 480.69±0.28kcal/100g with the addition of Bambara groundnut flour which has a relatively higher amount of fat. Significant differences ($p<0.05$) exist in the energy content of the cookie samples. Consumption of 100 g of the composite cookie in which BG is blended at 40% for instance would contribute about 22% of the 2100 kcal per day required for a healthy adult individual (468.72/2100 x 100/1 = 22.32%).

4.9 Mineral and Beta Carotene Composition of Cookie Samples

The result of the mineral analysis of the cookie samples are shown in Table 4.9. The calcium content of the cookies ranged from 241.36±0.02 to 272.62±0.02mg/100g. There was significant ($p<0.05$) difference in the calcium content of the cookies except for sample Bc which was not significantly ($p>0.05$) different from samples Cc and Dc. The calcium content increased with increased proportion of cassava flour. This observation may be attributed to the fact that Cassava is reasonably rich in calcium (Charles *et al*., 2005). Honi (2016) also observed a decrease in calcium with increased proportion of Bambara flour in a prepared snack. Calcium is an important mineral required for bone formation, blood clotting and muscle contraction (Onwuzuruike *et al*., 2020). Calcium deficiency leads to bone weakness (osteopenia) and fractures (osteoporosis); its excessive intake leads to hypercalcemia (metabolic alkalosis and loss of kidney function) and kidney stone formation (Institute of Medicine 2006; Kobue-lekalake *et al*., 2022). The recommended adequate intake of calcium for a child of 1–3 years is 500 mg/day (Institute of Medicine 2006), and considering 70% calcium bioavailability (WHO/FAO 2004), the consumption of 100g of the PVAC60%BG40% formulated cookie can contribute 32.7% (233.21 × 70/100 = 163.25; then, 163.25/500 × 100 = 32.65%) of calcium for the daily intake.

Table 4.9 Mineral Composition and β-carotene content of Cookies from Flour Blends of pro-vitamin A cassava and Bambara groundnut

Sample Code (PVAC:BG) (%)	Calcium (mg/100g)	Sodium (mg/100g)	Magnesium (mg/100g)	Phosphorus (mg/100g)	Potassium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	B-carotene (µg/100g)
Ac (100:0)	272.62±0.02^{e}	36.07±0.01^{f}	72.14±0.01^{b}	353.13±0.05^{a}	182.12±0.01^{a}	1.44±0.03^{a}	0.82±0.01^{a}	91.39±1.64^{c}
Bc (80:20)	268.21±1.40^{cd}	31.32±0.01^{e}	70.71±0.01^{a}	406.02±1.43^{b}	194.72±0.01^{b}	3.14±0.01^{b}	1.60±0.34^{b}	90.40±0.16^{c}
Cc (60:40)	269.68±0.72^{d}	24.05±0.01^{d}	79.14±0.01^{c}	437.12±7.75^{c}	201.03±0.02^{c}	4.22±0.02^{c}	2.84±0.06^{c}	85.73±0.12^{c}
Dc (40:60)	266.72±0.03^{c}	21.23±0.01^{c}	80.41±0.02^{d}	484.56±0.69^{d}	209.72±0.01^{d}	6.52±0.02^{d}	3.65±0.01^{d}	76.75±8.06^{b}
Ec (80:20)	249.92±0.01^{b}	19.82±0.01^{b}	104.14±0.01^{e}	527.86±0.02^{e}	223.18±0.01^{e}	9.88±0.02^{e}	5.55±0.01^{e}	75.44±0.46^{b}
Fc (0:100)	241.36±0.02^{a}	19.15±0.01^{a}	107.05±0.02^{f}	596.43±0.01^{f}	273.13±0.02^{f}	14.28±0.37^{f}	6.84±0.16^{f}	47.06±0.05^{a}

Values are Means ± standard deviation of duplicate determinations $^{a-f}$ Means with the same superscripts within the same columns are not significantly ($p>0.05$) different. Where PVAC: Pro-vitamin A Cassava flour, BF: Bambara groundnut flour

The sodium content of the cookies ranged from 19.15±0.01 to 36.07±0.01mg/100g. The sodium content was found to decrease with increased Bambara substitution. There was significant ($p<0.05$) difference in the sodium content of the cookies. The composite cookies recorded relatively low sodium content. The low sodium content of the cookies might be beneficial since low sodium diet has been reported to be beneficial in the prevention of high blood pressure (Onwuka, 2018).

The magnesium content of the cookies ranged from 72.14±0.01 to 107.05±0.02 mg/100g with sample Fc having the highest value and Ac the least value respectively. There was significant ($p<0.05$) difference in the magnesium content of the cookies. Increasing the proportion of Bambara flour resulted to an increase in the magnesium content of the composite cookies. The values obtained suggested that the consumption of every 100 g of the cookie would meet the FAO/WHO recommended daily intake of magnesium for infants and children (26 to 100 mg/day). However, the values obtained are below the recommended nutrient intake for adolescents (230 mg/day for females and 220 mg/day for males) and adults (220 mg/day for females and 260 mg/day for males) respectively (FAO/WHO, 1998).

The phosphorus content of the cookies ranged from 353.13±0.05 to 596.43±0.01mg/100g. Sample Fc (100% Bambara flour cookie) had the highest phosphorus content (596.43 mg/100g) while Sample Ac (100% Provitamin A cassava flour cookie) had the lowest. There was significant ($p<0.05$) difference in the phosphorus content of the cookies. The phosphorus content of the cookie increased with increased Bambara flour substitution. Phosphorus helps in bone growth, proper kidney function and cell growth (Musah *et al*., 2021). The increased phosphorous content implied that the mineral content of food products might be improved by developing composite products.

Potassium is a very important mineral for the proper functioning of all cells, tissues, and organs in the human body. It plays a key role in skeletal and smooth muscle contraction, making it important for normal digestive and muscular function (Onwuzuruike *et al*., 2020). Potassium also plays a role in maintaining the body's acid-alkaline balance (Musah *et al*., 2021). The potassium content of the cookies ranged from 182.12±0.01 (Ac) to 273.13±0.02mg/100g (Fc). There was significant ($p<0.05$) increase in increased substitution with Bambara flour. The values suggested that the potassium content of the cookie samples is quite high and might be good sources of potassium.

The iron content of the cookies ranged from 1.44±0.03 to 14.28±0.37mg/100g. Sample Fc (100% Bambara flour cookie) had the highest iron value (14.28mg/100g) which implied that the

development of composite cookies from Bambara groundnut and Provitamin A cassava flours improved the iron content of the final product. Blending with BG at 40% showed an increase of 362.8% (3.98-0.86/0.86 x 100/1) iron content from the control sample A; this is because iron content was found to be high in BG as 5.45 mg/100g by Abdualrahman *et al*., (2019) and 8.8 mg/100 g by Semba *et al*., (2021). Iron deficiency anemia is a serious problem for certain at-risk groups (Food and Nutrition Board, Institute of Medicine, 2002) and continued research is ongoing for the development of ready-to-eat foods with appreciable iron content. The recommended dietary allowance (RDA) of iron for a child of 1–3 years is 7 mg/day (Institute of Medicine, 2006). The iron bioavailability from plant-based diets can range from 5 to 10% (WHO/FAO, 2004), and assuming 10% bioavailability, the consumption of 100g of the PVAC60%BG40% formulated cookies can supply about 5.7% (3.98 × 10/100 = 0.398; then, 0.398/7.0 × 100 = 5.69%) of iron for a child aged 1–3 years old. This shows that, even though there is some improvement in the composite cookie in terms of iron supply, other diet sources rich in iron are required for adequate iron intake along with this cookie.

The zinc values of the cookies ranged between 0.82±0.01 to 6.84±0.16 mg/100g with sample Fc having the highest value while Ac having the least. The addition of the Bambara flour increased the zinc value of the cookie samples. This is because the zinc content of Bambara is reported to be high (2.14 to 19.73mg/100g) (Halimi *et al*., 2019). Zinc is an important mineral for growth and development, and also appears to improve immune function (Onwuzuruike *et al*., 2020). At PVAC60%BG40%, the zinc content of cookies was increased by 190.8% from the control (100% PVAC cookie sample). The RDA of zinc for a child of 1–3 years is 3.0 mg/day (Institute of Medicine, 2006). Considering a moderate zinc bioavailability diet (30% bioavailability that takes phytate zinc binding into account) (WHO/FAO, 2004), the consumption of 100g of PVAC60%BG40% formulated cookies would supply about 22.1% of the zinc (2.21 × 30/100 = 0.66 mg; then, 0.66/3×100=22.1%) for the daily requirement. The mineral contents of the formulated cookies all showed significant ($p<0.05$) differences for all the analysed minerals.

The level of β-carotene in the prepared cookies are revealed in Table 4.9. β- carotene content ranged from 47.06±0.05 (Fc) to 91.39±1.64µg/100g (Ac). There was no significant ($p>0.05$) difference in the beta carotene content among cookie samples Ac, Bc and Cc and also no significant ($p>0.05$) difference between samples Dc and Ec. Other samples however showed

significant difference. This study showed that an increase in the PVAC flour increased the level of β-carotene in the cookies. These findings agree with Okoye and Ezeugwu, (2019) and Ubbor *et al*., (2022) who reported that more PVAC flour added in foods increases the β-carotene content. This is plausible because crops that have pro-vitamin A have proved to be an effective means to alleviate vitamin A deficiency (Tumuhimbise *et al*., 2013; Hailu 2016). Among the pro-vitamin A carotenoids, beta-carotene is known to impart high vitamin A activities (12 µg beta-carotene = 1 µg retinol activity equivalent (RAE) (Kobue-lekalake *et al*., 2022)**.** Therefore, adequate intake of beta-carotene and other carotenoids are vital for normal eye vision, immune functions to fight infectious diseases and gene regulation, and as an antioxidant in the protection of cell components (DNA, RNA, proteins, and lipids) from oxidative stress damage (Böhm *et al*., 2020).

Moreover, the β-carotene is a powerful antioxidant that provide protection against oxidative processes in food systems The antioxidant activity of β-carotene has been attributed to their polyene frameworks (Mulindwa *et al*., 2019). This implies that the formulated cookies might have an advantage of improved shelf life.

4.10: Anti-nutritional Composition of Cookies

The levels of antinutritional factors found in the cookies are shown in Table 4.10. The results of hydrogen cyanide ranged from 0.02±0.00 to 0.14±0.00mg/100g with cookie sample Ac having the highest. The results showed that hydrogen cyanide obtained in the formulated samples was solely contributed by the provitamin A cassava flour since the hydrogen cyanide in the cookie sample Ac (100% provitamin A cassava flour cookie) was the highest and significantly ($p<0.05$) different from others. Increasing proportion of cassava flour resulted into increased hydrogen cyanide content in the formulated cookies. However, the hydrogen cyanide content of the cookies was below the lethal those of >10 mg/kg as stipulated by Codex Alimentarius Commission (2013).

The tannin content ranged from 0.04±0.01 to 0.21±0.01 mg/100g. Increasing proportion of BG flour resulted into increased tannin content in the cookies. Tannins reduce the protein digestibility by inhibiting the proteolytic activity and/or by forming indigestible complexes with dietary protein (Yao *et al*., 2015). However, the tannin in the cookies were generally below the reported lethal dose of 90 mg/100g (Ifie and Emeruwa, 2011). Therefore, the tannin level in the cookies might not influence digestion negatively since high tannin concentration impairs microbial enzyme activities including cellulose and intestinal digestion may be depressed (Onwuzuruike *et al*., 2020).

Table 4.10: Anti-nutritional Composition of Cookies (mg/100g)

Cookie Samples	Hydrogen cyanide	Tannin	Phytate
Ac	0.14±0.00[d]	0.04±0.01[a]	0.54±0.01[e]
Bc	0.12±0.00[c]	0.05±0.00[b]	0.53±0.00[e]
Cc	0.07±0.01[b]	0.07±0.01[c]	0.48±0.01[d]
Dc	0.07±0.01[b]	0.08±0.00[d]	0.42±0.01[c]
Ec	0.04±0.01[a]	0.12±0.01[e]	0.39±0.01[b]
Fc	0.02±0.00[a]	0.21±0.01[f]	0.32±0.01[a]

Values are means ± standard deviation of duplicate determination

Mean Values within the same column followed by different superscripts are significantly different ($P<0.05$).

From human health point of view, lowering the level of tannins in foods reduces the risk of bowel irritation, kidney irritation, liver damage, irritation of the stomach and the gastrointestinal pain (Adegbehingbe, 2015; Abdualrahman *et al*., 2019).

The phytate content of the cookies ranged from 0.32±0.01 to 0.54±0.01mg/100g which was lower than 25 mg/100g, the amount considered lethal to health (Onwuzuruike *et al.,* 2020). Increasing proportion of cassava flour resulted to increased phytate content in the cookies. Phytate is often considered as an antinutrient because it binds minerals (Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+}) in the digestive tract, making them unavailable (Dick *et al.,* 2018). It reduces mineral absorption thereby reducing bioavailability (Gupta *et al.,* 2013). According to Mune *et al*., (2011), phytic acid forms complexes with proteins, amino acids and trace minerals and therefore its reduction is important for improving the nutritional quality of foods.

The values obtained appeared to be low and in reasonable agreement with those reported by commonly consumed food articles (Okpala and Okoli, 2011; Onwuzuruike *et al.,* 2020).

As seen, the contents of anti-nutrients of the flour blends prior to cookie production were significantly ($P < 0.05$) higher than that of the cookies. The baking process (Zaroug *et al*., 2014) proved to reduce the anti-nutritional factors. Reduction of the anti-nutritional factors is a key to improvement in the nutritional quality of the cookies with respect to its protein digestibility.

4.11 Sensory Evaluation of Cookies

Table 4.11 shows the sensory evaluation results. The appearance score of the cookies ranged from 7.15-7.80 with which sample Ac was rated higher by the panelist than other cookies hence had the highest score (7.80) and Fc had the lowest (7.15). This could be due to the fact that the increasing addition of Bambara caused the cookies to turn darker than cookies containing higher proportion of cassava flour. This is plausible since the darkening/browning is an expected effect of caramelization and Maillard reactions, and the higher proteins contributed by Bambara flour must have reacted better with sugar during baking (Manzocco *et al.,* 2011; Nwadi *et al.,* 2020). However statistically, no significant ($p>0.05$) difference was observed up to 100% blending with Bambara flour substitution in terms of appearance as they were all liked moderately by the panelists.

Taste is the primary factor that determines the acceptability of any food product which has the highest impact as far as market success of product is concerned (Abayomi *et al.,* 2013).

Table 4.11: Sensory Evaluation of Cookies Prepared from Pro-vitamin A cassava (PVAC) and Bambara groundnut (BG) blends

Sample Code (PVAC:BG) (%)	Appearance	Taste	Aroma	Crispness/Texture	Overall Acceptability
Ac (100:0)	7.80±0.83^{a}	7.35±0.93^{d}	7.00±1.49^{a}	6.60±1.31^{a}	7.70±0.66^{c}
Bc (80:20)	7.40±1.05^{a}	7.30±0.98^{d}	6.90±1.59^{a}	6.70±1.34^{a}	7.40±0.88^{c}
Cc (60:40)	7.55±0.61^{a}	6.75±0.91^{cd}	6.40±1.35^{a}	6.40±1.27^{a}	7.15±0.67^{c}
Dc (40:60)	7.45±0.83^{a}	6.55±0.89^{c}	6.40±1.27^{a}	6.20±1.40^{a}	6.50±0.89^{b}
Ec (20:80)	7.45±1.10^{a}	5.80±1.01^{b}	6.60±1.10^{a}	6.40±1.57^{a}	5.85±1.04^{a}
Fc (0:100)	7.15±1.18^{a}	5.05±0.91^{a}	6.40±1.31^{a}	6.40±0.88^{a}	5.50±1.24^{a}

Mean values within the same column followed by different superscripts are significantly ($p<0.05$) different.

The preferences for taste of the samples showed a significant ($p<0.05$) decrease in increasing substitution of Bambara flour in the cookies. Sample Ac and Bc were liked moderately by the panelists, Cc and Dc were liked slightly while samples Ec and Fc were neither liked nor disliked. Sample Ac was the most preferred in taste (7.35) while Fc was the least preferred (5.05). This could be attributed to the fact that most biofortified yellow-flesh cassava variants are sweet tasting, containing mild-to-moderate toxic cyanogenic glucosides compared to the majority of white-flesh variants (Talsma, 2014).

The aroma of the cookies ranged from 7-00-6.40 from sample Ac to Fc respectively with Ac exhibiting better aroma than other samples as rated by the panelist and was liked moderately while other cookie samples were liked slightly by the panelists. However, there was no significant ($p>0.05$) difference in aroma among the samples.

The crispness/texture of the cookies decreased with increase in Bambara flour ranging from 6.60 to 6.40. Cookie sample Ac had the highest crispness 6.60 while Fc sample had the lowest score for crispness. It was observed that crispness reduced with increased Bambara flour substitution. It might be implied that the presence of Bambara flour affected the crispiness of the cookies. The slight decrease in crispness with increase in Bambara flour substitution may due to the effect of the total fat content of Bambara which is about 6.6% (Arise and Malomo, 2021). Abayomi *et al.*, (2013) also attributed decrease in crispness to increased fat content of Soybean. On the contrary, Eke-Ejiofor *et al.*, (2023) in a recent study reported higher mean scores for crispness of cookies with increased Bambara groundnut substitution which was attributed to the higher fat content of the Bambara groundnut flour compared to cassava flour. The improved crispness/texture associated with cookies with higher proportion of cassava flour in this study might be attributed to the cohesiveness of starch matrix present in cassava flour. Similar observation was recorded by Onwuzuruike *et al*., (2020). However, there was no significant ($p>0.05$) difference observed as the crispness of all the cookie samples were liked slightly by the panelist.

The results of overall acceptability showed that samples Ac, Bc, and Cc were moderately accepted while sample Dc was slightly accepted by the panelist with ratings 7.70, 7.40, 7.15 and 6.50 respectively. Sample Ec and Fc were neither liked nor disliked by the panelists with ratings 5.85 and 5.50 respectively. It was noted that the overall acceptability was determined by increase in Bambara flour and cookies samples up to 40% Bambara substitution were equal to or greater than

value of like moderately (7). It was therefore concluded that cookies with substitution up to 40% level of Bambara could be baked with satisfactory performance and moderate acceptance.

4.12 *In-Vitro* Protein Digestibility of Cookies

Protein digestibility is one of the major protein's quality determinants. Decrease in pH during the digestion process is an indication of increased protein digestibility which is attributed to the release of hydrogen ions by the hydrolyzed peptide bonds in the protein molecule (Falade and Akeem, 2020). Fig. 4.1 shows the *in-vitro* protein digestibility of the formulated cookies. The percentage *in-vitro* protein digestibility ranged from 76.05±0.04 to 81.06±0.01% with cookie sample Ac having the least value and Fc the highest value respectively. Significant ($P < 0.05$) increases were observed with increased BG flour substitution. The increase in *in-vitro* protein digestibility of the fortified cookies might be ascribed to incorporation of the Bambara groundnut protein that is characterized by high digestibility (Abdualrahman *et al*., 2019) compared with cassava protein. Chinma *et al*., (2012) also attributed increase in *in-vitro* protein digestibility of formulated cookies to increased protein content.

The relatively high protein digestibility of the selected cookies shows that the cookies are highly digestible and may be attributed to the low levels of tannins found in the cookies; since dietary tannins are often responsible for the poor digestibility of dietary proteins (Okpala and Okoli, 2011). Also, the elimination of phytic acid contributes to the improvement in protein digestibility (Olanipekun *et al*., 2015) especially with regards to the soaking/natural fermentation which was carried out in the process of production of BG flour the main source of protein in the cookies. Oyeyinka *et al*., (2019) also attributed increase in in-vitro protein digestibility of cooked Bambara grains to decrease in tannin and phytic acid contents. Cooking methods like baking can also improve the digestibility of foods. Kiers *et al. (*2000) reported that digestibility of cereals and legumes increased during cooking and fermentation. This could be attributed to the partial degradation of complex storage proteins into more simpler and soluble products (Olanipekun *et al*., 2015). The percentage *in-vitro* protein digestibility obtained in this study were comparable to the range 75.07 to 79.17% recorded for Bambara-based bread meal (Abdualrahman *et al.*, 2019)

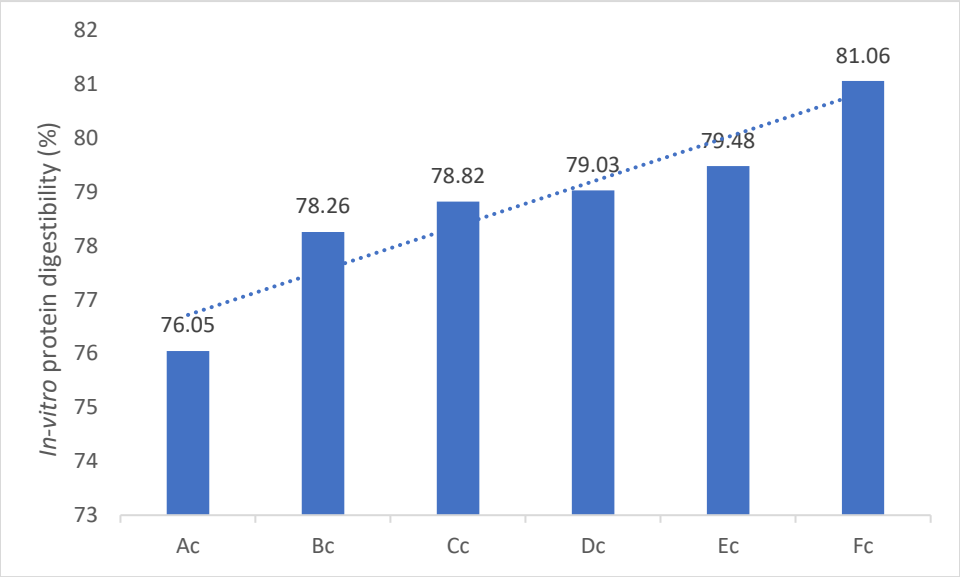

Figure 4.1: *In-vitro* protein digestibility of cookie samples

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The findings of this research revealed that the cookies produced with Bambara flour substitution up to 40% was moderately acceptable and nutritionally superior to that of the whole cassava flour and whole Bambara flour cookies respectively. The high protein content in the provitamin A-Bambara supplemented cookies would be of nutritional importance in most developing countries like Nigeria where many people can hardly afford high proteinous foods because of their high cost.

The economic impact of utilization of flour produced from indigenous crops (provitamin A cassava-Bambara flour) will enhance gross domestic products in Nigeria and bring about reduction in foreign exchange on wheat importation and thus enhance the industrial utilization of local crops It can also be suggested in the snacking pattern of children and adults in Nigeria

In addition, Incorporation of provitamin A cassava in ready to eat foods could diversify food intake and ensure food and nutrition security. This cookie will be highly appreciated by people having celiac diseases as this is a gluten-free cookie.

5.2 Recommendations

This study recommends that cookies produced from provitamin A cassava and Bambara flours be improved and optimized for the benefit of individuals with celiac diseases, as well as protein energy malnutrition. The formulated cookies require assessment of shelf stability.

Even though, yellow root cassava has high provitamin A and non-provitamin A carotenoid than white fleshed cassava, in Nigeria, consumption of yellow root cassava for food is lower compared to white fleshed cassava. Therefore, there is a need to promote yellow root cassava by developing more sensory acceptable products. More foods with good fat content should consider utilization of provitamin A cassava to enhance bioavailability. All stakeholders who involve in combating VAD in the country should involve and take part to promote and provide nutrition education on the benefits of yellow root cassava. Agricultural sector should play role to enhance the production and accessibility of yellow root cassava with high β-carotene content.

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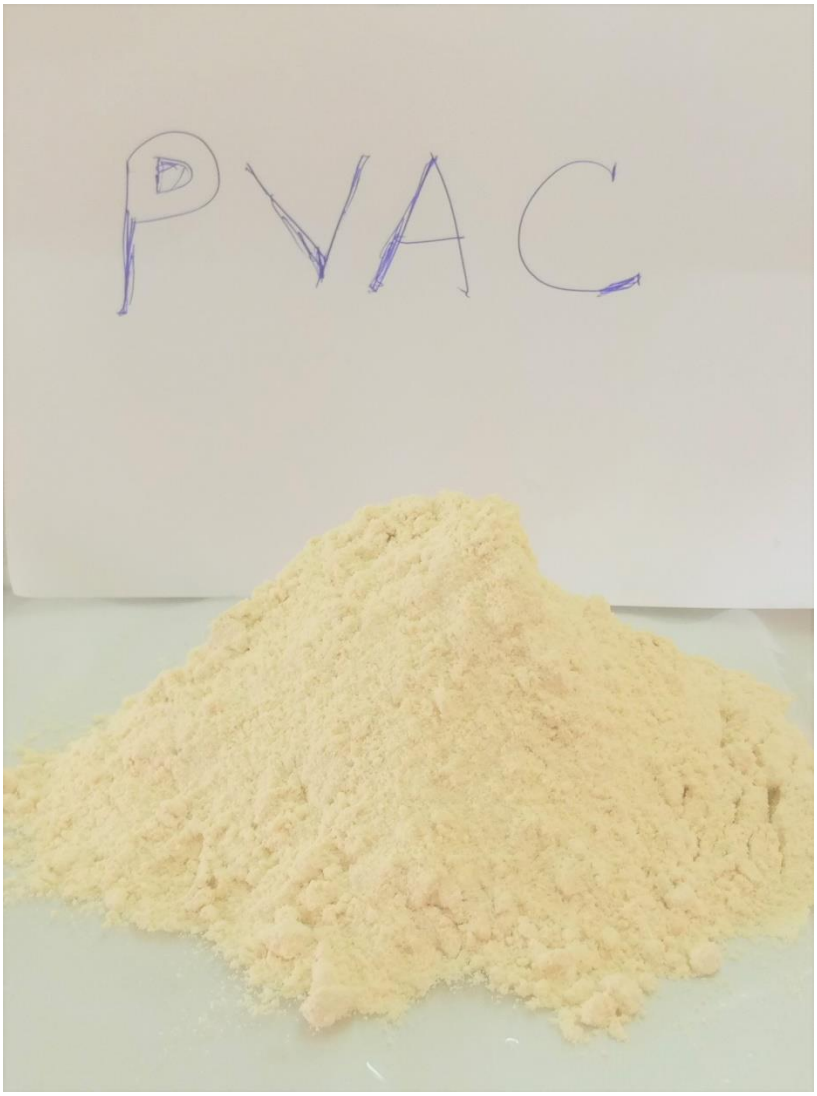
APPENDIX

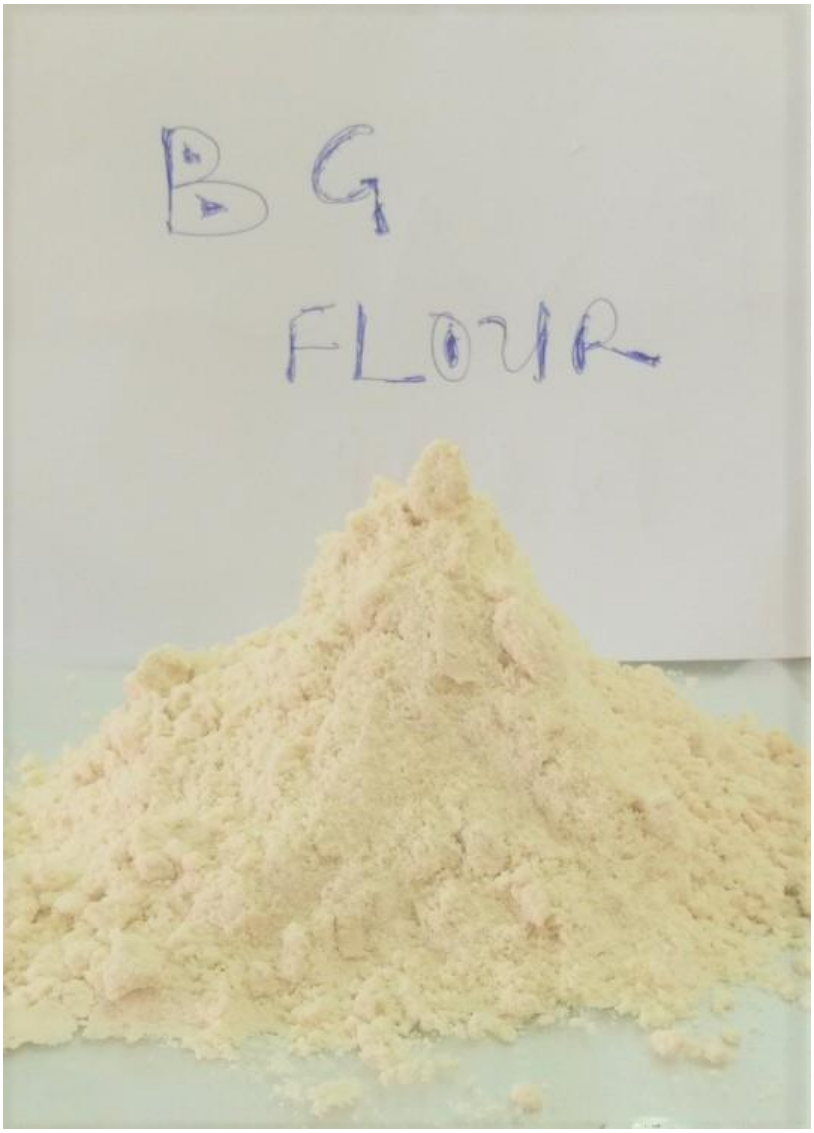
Appendix 1: Peeled and Washed Pro-vitamin A cassava variety [IBA48100 (Security)]

Appendix 2: Dehulled Bambara groundnut

Appendix 3: Provitamin A cassava (PVAC) flour



Appendix 4: Bambara groundnut (BG) flour



Appendix 5: Cookie sample Ac=305 (100:0)

Appendix 6: Cookie sample Bc=311 (80:20)

Appendix 7: Cookie sample Cc=817 (60:40)

Appendix 8: Cookie sample Dc=502 (40:60)

Appendix 9: Cookie sample Ec=104 (20:80)

Appendix 10: Cookie sample Fc=174 (0:100)

Appendix 11: Sensory Evaluation Form

Panelist No…… Sex……

Age group (a) 10-20 years (b) 20-30 years (c) 30 - 40 years (d) above 40 years

Time…….. Date…….....

Education level (a) Bachelor degree (b) Master's degree (c) others: specify……

Please look and taste each of the (6) coded cookie samples. You are provided with a sachet of potable water to rinse your mouth after tasting each sample.

Indicate how much you like or dislike each sample by checking the appropriate sample attribute and indicate your preference (1-9) in the column against each attribute. Put the appropriate number against each attribute.

9 – Like extremely
8 – Like very much
7- Like moderately
6- Like slightly
5- Neither like nor dislike
4- Dislike slightly
3- Dislike moderately
2- Dislike very much
1- Dislike extremely

Attributes	Sample codes					
	305	311	817	502	104	174
Apperance						
Taste						
Aroma						
Crispness/Texture						
Overall Acceptability						
Would you prefer to buy a product?	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No	Yes/No

Comments (optional)

……

……

……

Thank you.

Appendix 12: ANOVA of Sensory Evaluation Result

ANOVA

				Sum of Squares	df	Mean Square	F	Sig.
Appearance	Between Groups	(Combined)		4.467	5	.893	.985	.430
		Linear Term	Contrast	2.926	1	2.926	3.226	.075
			Deviation	1.541	4	.385	.425	.791
	Within Groups			103.400	114	.907		
	Total			107.867	119			
Taste	Between Groups	(Combined)		80.267	5	16.053	18.013	.000
		Linear Term	Contrast	74.983	1	74.983	84.134	.000
			Deviation	5.284	4	1.321	1.482	.212
	Within Groups			101.600	114	.891		
	Total			181.867	119			
Aroma	Between Groups	(Combined)		7.367	5	1.473	.796	.555
		Linear Term	Contrast	4.346	1	4.346	2.348	.128
			Deviation	3.021	4	.755	.408	.803
	Within Groups			211.000	114	1.851		
	Total			218.367	119			
Crispness	Between Groups	(Combined)		3.100	5	.620	.360	.875
		Linear Term	Contrast	1.260	1	1.260	.731	.394
			Deviation	1.840	4	.460	.267	.899
	Within Groups			196.600	114	1.725		
	Total			199.700	119			
OA	Between Groups	(Combined)		77.867	5	15.573	18.474	.000
		Linear Term	Contrast	75.911	1	75.911	90.051	.000
			Deviation	1.955	4	.489	.580	.678
	Within Groups			96.100	114	.843		
	Total			173.967	119			